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Review Article

# Liposomally targeted cytotoxic drugs for the treatment of cancer

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## Introduction

Phospholipid spherules composed of lipid bilayer membranes entrapping a central aqueous core were first described more than 30 years ago (Bangham et al 1965). The term liposome was coined in 1968 (Sessa & Weissmann 1968) and the first suggestions that these vesicles might have potential as vehicles for targeted drug delivery for a range of diseases, including cancer, appeared shortly afterwards (Gregoriades et al 1974; Gregoriades 1976a, b). However, the process of turning this expectation into a clinical reality has suffered a number of setbacks and has taken more than a quarter of a century. In the process, new types of liposomes with favourable in-vivo pharmacokinetics and biodistribution patterns have been generated (Lasic & Papahadjopoulos 1995). Many of these preparations have been subjected to extensive examination and an increasing number of agents have entered clinical trials. In this review, we will trace the development of those liposomes that are currently undergoing (or are about to undergo) clinical evaluation.

## The mechanism of tumour targeting by liposomes

The ability of liposomes to localize effectively to tumours is a somewhat enigmatic property. Even in the absence of tumour cell-specific ligands attached to their surface, liposomes will localize to tumours. In this sense, it is generally accepted that liposomal tumour targeting is a passive function and depends largely on the number of times that an individual liposome passes through the vascular network within a tumour. It is well recognized that the blood vessels in a tumour are abnormally leaky as a result of significant structural and functional anomalies. This leakiness and the co-existing lack of a fully functional system of lymphatic drainage are thought to account for the extravasation and retention of liposomes within the tumour interstitium. Therefore, by altering the physicochemical properties of a liposome, its ability to remain in the circulation (and hence its likelihood of being deposited in the tumour) can be altered. Attempts to exploit this physicochemical property of liposomes underlie the development of so-called long-circulating liposomes with extended circulation half-lives (see below).

## The history of liposome development

The early development of liposomal therapeutics was beset by a number of problems (Martin 1997). Formidable difficulties were presented by the need to produce stable drug-containing liposomes in a reliable, reproducible way. The entrapment conditions for any particular agent need to be optimized individually. Because liposomes can carry drugs in one of three potential compartments (water-soluble agents in the central aqueous core, lipid-soluble agents in the membrane, peptides and small proteins at the lipid–aqueous interface), a diverse range of optimal encapsulation conditions may exist for different agents. In addition, the release kinetics of the entrapped agents can vary, depending on the liposomal formulation, and this can affect the therapeutic efficacy. Therefore, development of agents for pre-clinical and clinical use can be both laborious and expensive. In early in-vivo studies, liposomes were shown to have very

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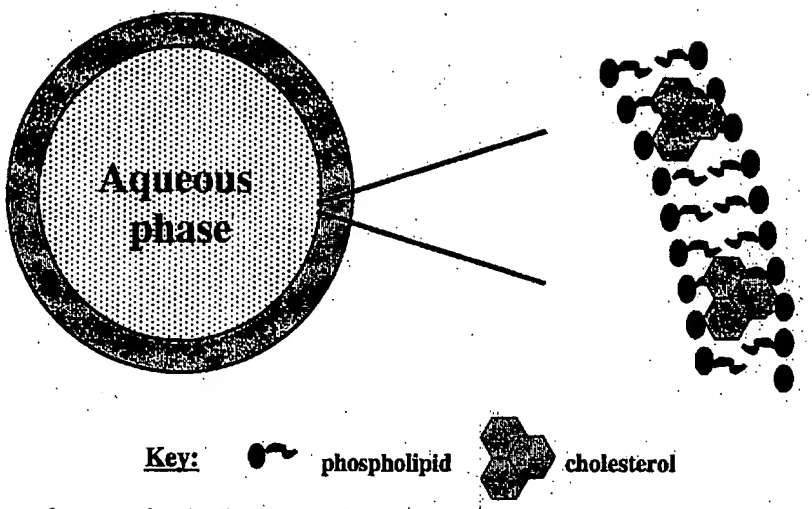


**Figure 1** Schematic diagram of a conventional unilammelar vesicle. The magnified area shows the organization of the lipid bilayer membrane, which is composed of phospholipid molecules and cholesterol. The aqueous core represents a reservoir of the entrapped drug.

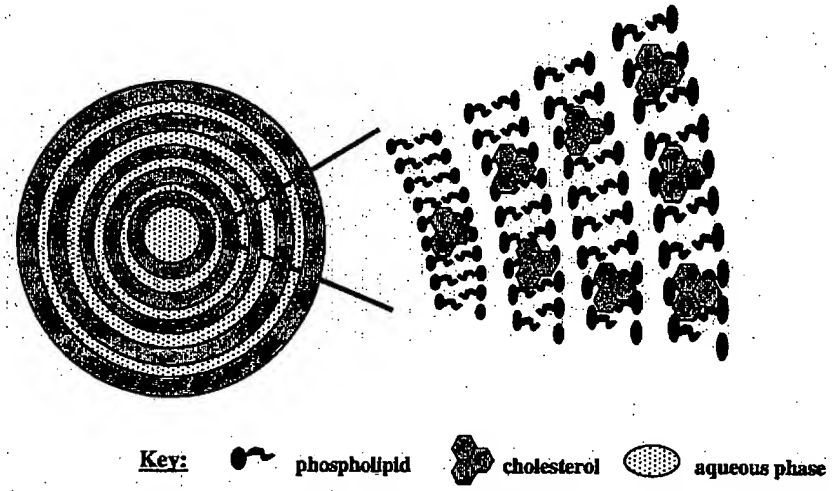


**Figure 2** Schematic diagram of a conventional multilammelar vesicle. The magnified area shows the organization of the multiple repeated lipid bilayer membranes composed of phospholipid molecules and cholesterol. The aqueous phase occupies the space between the lipid layers. Lipophilic drugs can be entrapped in the lipid membranes and hydrophilic drugs can be contained in the aqueous phase.

short circulation half-lives owing to rapid liposomal opsonization by plasma proteins and phagocytosis by fixed tissue macrophages of the reticuloendothelial system (RES). In addition, lipid exchange with plasma lipoproteins can destabilize liposomes and lead to their rupture, with rapid release of the entrapped drug. Taken together, these various problems meant that the available liposomal formulations offered few advantages over the administration of the unencapsulated drug. As a result, the early clinical applications of liposomes were largely limited to situations in which targeting of the RES was advantageous (e.g. treating systemic protozoal and fungal infections) (Adler-Moore 1994; Ng & Denning 1995; Russo et al 1996; Prentice et al 1997).

The first breakthrough in the problem of the rapid clearance of liposomes from the circulation came from studies correlating liposome permeability, membrane fluidity and liposome size with the circulation half-life (Senior & Gregoriades 1982a, b). These studies provided the background for the development of some of the currently available conventional liposomes. In the search for liposomes with improved pharmacokinetic parameters, a number of groups have evaluated the effect of adding various components to the lipid membrane. This work has been driven, in part, by the discovery that the stability of red cells in the blood is mediated by their hydrophilic, sialic acid-rich glycocalyx (Durocher et al 1975). By incorporating different purified glycolipids, a new class of so-called sterically stabilized liposomes has been generated and the pharmacokinetics and biodistribution of these agents have been evaluated in-vivo in murine models (Allen & Chonn 1987). Significant enhancement of circulation half-life was achieved with a ganglioside (monosialoganglioside GM1) extracted from bovine brain tissue. This agent was deemed

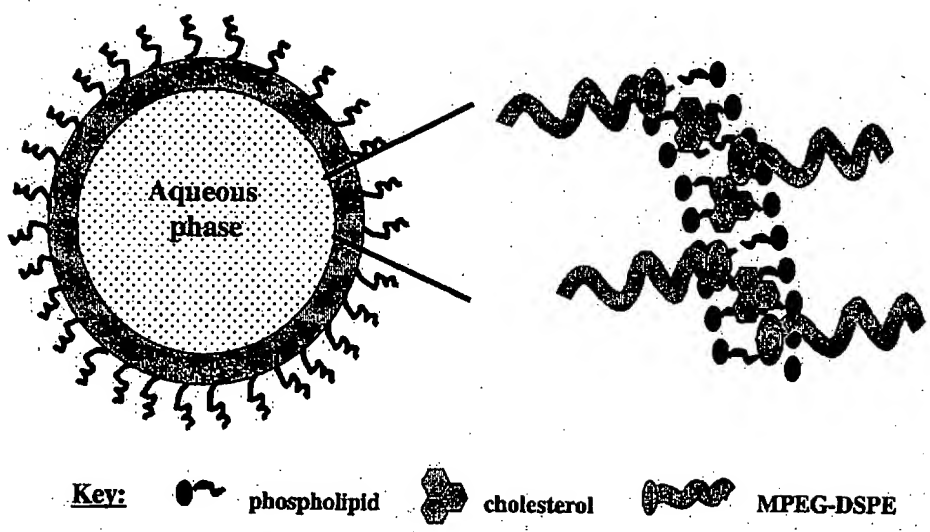


**Figure 3** Schematic diagram of a sterically stabilized liposome. The magnified area shows the organization of the lipid bilayer membrane and the disposition of the pegylated distearoyl phosphatidylethanolamine (MPEG-DSPE) polymer molecules, which are attached to the membrane by a lipophilic fatty acyl anchor and protrude from both the internal and external faces of the membrane. Only the external MPEG-DSPE molecules are represented in the schematic of the entire liposome. Drugs can be entrapped within the aqueous core of such liposomes.

to be unsuitable for clinical use, a decision that has assumed added significance in the wake of the appearance of bovine spongioform encephalopathy and new variant Creutzfeld-Jakob disease. At about the same time, hydrogenated phoshphatidylinositol (HPI), a molecule extracted from soybean oil, was shown to achieve similar results to GM1 (Gabizon & Papahadjopoulos 1988). Unfortunately, the extraction of HPI proved to be prohibitively expensive and its development was halted. A significant advance came with the discovery that liposomes with methoxypolyethylene glycol (MPEG)-derivatized lipids in their membranes had prolonged circulation half-lives and could be readily produced in large quantities (Klibanov et al 1990; Allen & Hansen 1991; Papahadjopoulos et al 1991). MPEG is thought to act by providing a steric barrier against interactions with plasma proteins, such as opsonins and lipoproteins, and cell surface receptors, such that pegylated liposomes evade clearance by the RES (Gabizon 1994). Subsequent research effort has helped to define the optimal formulation (including choice of MPEG derivative) of such liposomes (Lasic 1996).

Although the polyethylene glycol (PEG) barrier may have beneficial effects in terms of extending the circulation half-life and increasing the area under the curve of drug exposure, there is some evidence that its presence may impede drug release/delivery to the target cell population. Attempts have been made to solve this problem by generating liposomes that are reversibly pegylated (Adlakha-Hutcheon et al 1999; Zalipsky et al 1999). One system uses PEG-phosphatidylethanolamine with acyl chains of varying lengths, which determine the rate of exchange of the PEG component out of the liposome membrane. In effect, such liposomes are fitted with a "timer" that determines how long they will remain functionally pegylated (Adlakha-Hutcheon et al 1999). An alternative approach uses MPEG attached to the liposome membrane by linkers that are cleavable under mildly reducing conditions, such as those found within the tumour milieu (Zalipsky et al 1999). Such studies are likely to represent just the beginning of a trend towards rational design of the components of the lipid membrane in order to influence favourably the behaviour of liposomes in-vivo.

## Classification of liposomes

There are a number of ways in which liposomes can be categorized. One scheme uses the simple physical characteristic of lamellarity (the number of phospholipid membrane layers) to divide liposomes into unilamellar vesicles (ULV) (Figure 1) and multilamellar vesicles (MLV) (Figure 2) (Perez-Soler 1989). ULV have a single phospholipid bilayer membrane and a diameter of 0.05–0.25 $\mu$m. These liposomes can be further split into small ULV (SULV) with a diameter of 0.05–0.1 $\mu$m and large ULV (LULV) with a diameter of 0.1–0.25 $\mu$m. Because ULV contain a large central aqueous compartment, they are ideally suited to the encapsulation of water-soluble agents. Passive drug encapsulation is a relatively inefficient means of loading these liposomes and, more frequently, a gradient-driven system is used to achieve efficient drug entrapment. MLV are composed of concentric phospholipid bilayer membranes in an onion-skin arrangement and have a diameter of 1–5 $\mu$m. In contrast to ULV, they only contain a small aqueous compartment (< 10%), which means that they preferentially entrap lipid-soluble drugs efficiently. This fact is likely to assume great importance in the future because a number of active cytotoxic drugs are highly lipid-soluble, a property that has sometimes hampered their clinical development (Leyland-Jones 1993).

An alternative classification scheme has been proposed (Martin 1997). This categorization draws on both physical and physiological features of the liposomal formulations.

**Table 1** Summary of pre-clinical biodistribution and pharmacokinetic studies using conventional liposomes in animal models.

| Liposome | Tracer | Tumour models | Comments | Reference |
|---|---|---|---|---|
| SUV | Doxorubicin | J-6456 (lymphoma) | There was decreased cardiac uptake with PS/PC/Chol and PC/Chol (but not DPG/PC/Chol) liposomes with no loss of antitumour efficacy | Gabizon et al (1982) |
| SUV | $^{111}$In-NTA | EMT6 | This study compared the tumour targeting achieved by various liposome formulations with neutral, positive and negative surface charge. The highest tumour and lowest RES uptakes were documented for neutral SUV composed of a 2:1 ratio of DSPC/Chol. There was significant RES uptake of all formulations, although this was lowest for neutral liposomes | Proffitt et al (1983) |
| SUV | Doxorubicin | J-6456 (lymphoma) | There was increased liver/spleen uptake with PS/PC/Chol liposomes compared with the unencapsulated drug. Increased drug levels were documented in J-6456 cells isolated from the liver after liposomal drug delivery | Gabizon et al (1983) |
| MLV | NDDP<br>CDDP | B16 (melanoma)<br>VX2 (hepatic cancer) | There was no difference in tumour uptake after intravenous injection of unencapsulated cisplatin or liposomal NDDP (MLV composed of a 7:3 ratio of DMPC/DMPG) in a B16 melanoma model. There was significantly greater tumour uptake after intravenous and intra-arterial injection of the liposomal as compared with the unencapsulated drug in the VX2 model | Khokhar et al (1988) |
| SUV | Vincristine | P388 (leukaemia)<br>L1210 (leukaemia) | A comparison between egg PC/Chol and DSPC/Chol liposomes containing vincristine was performed. The LD50 of the DSPC/Chol preparation was significantly lower than that of unencapsulated vincristine | Mayer et al (1990) |
| MLV | NDDP | None | The serum AUC after intravenous or intraperitoneal injection was significantly higher for liposomal NDDP compared with unencapsulated CDDP. The AUC in the peritoneum was greater after intraperitoneal liposomal NDDP compared with intraperitoneal unencapsulated CDDP | Vadiei et al (1992) |
| SUV | $^{111}$In-NTA<br>Daunorubicin | P-1798 (lymphosarcoma)<br>MA16C (breast) | Tumour deposition of $^{111}$In-NTA and daunorubicin were 2.5 to 20-fold greater for liposomal compared with unencapsulated agents in both the P-1798 and MA16C models. The tumour AUC was 10-fold greater for liposomal as compared with unencapsulated daunorubicin in the P-1798 model | Forssen et al (1992) |

**Table 1** (*cont*).

| Liposome | Tracer | Tumour models | Comments | Reference |
|---|---|---|---|---|
| SUV | Daunorubicin | P-1798 (lymphosarcoma) | There was a 2.5-fold increase in the tumour AUC of liposomal as compared with unencapsulated daunorubicin | Forssen et al (1996) |
| SUV | $^{67}$Ga- or $^{111}$In-NTA $^{67}$Ga- or $^{111}$In-deferoxamine $^{99m}$Tc-HMPAO | Mouse sarcoma 180 Ehrlich tumour | This study was designed to define the optimal size and phospholipid/cholesterol content for tumour targeting by SUV. For DSPC/Chol (ratio 2:1) SUV, the optimal size was found to be 80–250 nm. The optimal phospholipid was DSPC and the optimal ratio of DSPC/Chol was 1:1 or 2:1 | Ogihara-Umeda et al (1996) |
| SUV | Doxorubicin | B16/BL6 (melanoma) L1210 (leukaemia) | Maximum drug deposition was seen at 1 h and 48 h for unencapsulated and liposomal drugs, respectively. The tumour drug exposure was increased 2-3-fold (melanoma) and 10-fold (leukaemia), respectively, for liposomal as compared with unencapsulated doxorubicin | Harasym et al (1997) |
| SUV | Doxorubicin | C-26 (colon) | A comparison of liposomes composed of DSPC/Chol and DSPC/Chol/PEG-DSPE was performed. The plasma AUC was 2-fold greater for pegylated liposomes, but the tumour AUC over 72 h was 1.44-fold greater for conventional liposomes | Hong et al (1999) |

AUC, area under the curve; CDDP, *cis*-dichlorodiammine platinum (II); Chol, cholesterol; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; DPG, diphosphatidylglycerol; DSPC, distearoyl phosphatidylcholine; HMPAO, hexamethylpropylene-amine oxime; LD50, dose that causes death in 50% of animals; MLV, multilamellar vesicles; NDDP, *cis*-bis-neodecanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II); NTA, nitrilotriacetic acid; PC, phosphatidylcholine; PEG-DSPE, pegylated distearoyl phosphatidyl-ethanolamine; PS, phosphatidylserine; RES, reticuloendothelial system; SUV, small unilamellar vesicles.

The physical characteristic relates to whether the lipid membrane has been modified (so-called pure lipid (conventional) and surface-altered (sterically stabilized) liposomes). Idealized representations of the structures of conventional (ULV and MLV) and sterically stabilized liposomes are shown in Figures 1, 2 and 3, respectively. The physiological division describes the relative extent of uptake of the liposomes by the RES (so-called RES-targeted and RES-avoiding liposomes). The basic premise behind the use of RES-targeted liposomes is an attempt to deliver drugs with therapeutic efficacy against lesions in the RES (e.g. fungal infections) (Adler-Moore 1994; Ng & Denning 1995; Russo et al 1996; Prentice et al 1997), or to use the liposome as a form of slow-release vehicle from which the drug gradually leaks into the systemic circulation (Gokhale et al 1996). In contrast, RES-avoiding liposomes are designed to be delivered to their target tissue (tumour) and deliver their contents directly at the site of therapeutic activity. For the remainder of this review, we will focus on the fundamental difference between conventional and sterically stabilized liposomes with a view to pointing out their individual merits and disadvantages.

## Pre-clinical and clinical studies

The literature is replete with reports of the pre-clinical and, to a lesser extent, the clinical evaluation of a diverse range of liposomal agents. In this review, we will concentrate on those agents that have undergone more than just cursory clinical evaluation. In the interests of clarity, the data from biodistribution and pharmacokinetic studies will be considered separately from those derived from therapeutic studies.

### Pre-clinical biodistribution and pharmacokinetic studies

*Conventional liposomes*

Table 1 presents a summary of the published pre-clinical studies on the biodistribution and pharmacokinetics of

**Tabl 2** Summary of pre-clinical biodistribution and pharmacokinetic studies using sterically stabilized liposomes in animal models.

| Liposomes | Tracers | Tumour models | Comments | Reference |
|---|---|---|---|---|
| PC/Chol/HPI<br>PC/Chol/GM1 | $^{67}$Ga-deferoxamine | J-6456 (lymphoma) | Optimal tumour targeting was seen with 100-nm liposomes containing a neutral phospholipid and a negatively charged glycolipid (HPI or GM1). Blood (60-fold) and tumour (25-fold) levels were increased and RES levels were decreased (4-fold) | Gabizon & Papahadjopoulos (1988) |
| HPC/Chol/HPI<br>PG/PC/Chol | Doxorubicin | None | Clearance of doxorubicin was significantly slower for HPI-containing liposomes compared with conventional liposomes. Both liposomal formulations were associated with significantly reduced cardiac drug deposition | Gabizon et al (1989) |
| PG/PC/Chol<br>PG/DSPC/Chol<br>DPPG/DSPC/Chol<br>DSPC/Chol/HPI<br>DSPC/Chol/GM1 | $^{67}$Ga-deferoxamine<br>$^{111}$In-bleomycin | B16 (melanoma)<br>J-6456 (lymphoma)<br>LS174T (colon) | Optimal tumour localization was seen for liposomes composed of HPI/PC/Chol or GM1/PC/Chol. Tumour uptake values exceeded 10% ID $g^{-1}$ at 24 h. Radiolabelled bleomycin demonstrated a 20- to 40-fold increase in uptake for liposomal agent at 24 h | Gabizon et al (1990) |
| HPI/HPC/Chol<br>PG/PC/Chol | Doxorubicin<br>Epirubicin | J-6456 (lymphoma) | There was significantly increased tumour uptake of drugs in HPI-containing liposomes, but not conventional PG-containing liposomes. In malignant ascites, 10% of the administered drug was recovered after intravenous HPI-containing liposomes | Gabizon (1992) |
| DSPC/Chol<br>DSPC/Chol/GM1<br>DSPC/Chol/PEG-PE | $^{67}$Ga-deferoxamine | C-26 (colon) | The AUC in tumour tissue was increased 2- to 3-fold and the AUC in the RES was decreased 2-fold for pegylated compared with conventional liposomes | Huang et al (1992a) |
| Egg PC/Chol/GM1<br>Egg PC/Chol/MPEG-DSPE | Colloidal gold<br>Rhodamine B | C-26 (colon) | Electron microscopy demonstrated deposition of gold-containing liposomes in perivascular space in tumours. Colloid gold particles were seen in Küppfer cells, but not within liver parenchyma or tumour cells | Huang et al (1992b) |
| Egg PC/Chol/MPEG-DSPE | Colloidal gold | KS-like lesions | Extravasation and transcytosis of liposomes were significantly increased in KS-like dermal lesions compared with adjacent normal skin of transgenic mice bearing the human immunodeficiency virus *tat* gene | Huang et al (1993) |

**Table 2** *(cont).*

| Liposome | Tracers | Tumour models | Comments | Reference |
|---|---|---|---|---|
| HSPC/Chol/MPEG-DSPE | Doxorubicin | PC-3 (prostate) | Microfluorometry demonstrated 25-fold increase in the tumour AUC with liposomal compared with unencapsulated drug | Vaage et al 1994 |
| HSPC/Chol/MPEG-DSPE | Doxorubicin | MFH (sarcoma) | Increased drug deposition was seen in tumour compared with adjacent normal brain tissue with liposomal drug, but not unencapsulated drug. Peak tumour deposition of doxorubicin was 14-fold greater with the liposomal drug | Siegal et al (1995) |
| HSPC/Chol/MPEG-DSPE | Doxorubicin Texas Red | AsPC-1 (pancreas) | Microfluorometry demonstrated a 6- to 16-fold increase in tumour AUC for liposomal compared with unencapsulated drug. Diffusion of drug from perivascular liposomes to nuclei of stromal and tumour cells was demonstrated | Vaage et al 1997 |
| HSPC/Chol/MPEG-DSPE | Cisplatin | C-26 (colon) Lewis lung tumour | The tumour AUC was 28-fold higher for liposomal compared with unencapsulated cisplatin. There was a 4-fold reduction in renal drug deposition with the liposomal agent | Newman et al (1999) |
| HSPC/Chol/MPEG-DSPE | $^{111}$In-DTPA | KB (head and neck) | There was prolonged circulation of the radiotracer in liposomes compared with the unencapsulated agent with an approximately 10-fold increase in circulation half-life. Maximum tumour uptake was seen at 24 h ($5.5\pm3.0\%$ ID $g^{-1}$) and 5 min ($1.0\pm0.2\%$ ID $g^{-1}$) for encapsulated and unencapsulated radiolabel, respectively | Harrington et al (2000a) |
| HSPC/Chol/MPEG-DSPE | $^{111}$In-DTPA | KB (head and neck) | There was an inverse correlation between uptake of radiolabelled liposomes and tumour size with significant reduction of liposome uptake in areas of tumour necrosis | Harrington et al (2000b) |

AUC, area under the curve; Chol, cholesterol; DSPC, distearoyl phosphatidylcholine; GM1, monoganglioside; HPC, hydrogenated phosphatidylcholine; HPI, hydrogenated phosphatidylinositine; HSPC, hydrogenated soy phosphatidylcholine; ID, injected dose; KS, Kaposi's sarcoma; MPEG-DSPE, pegylated distearoyl phosphatidylethanolamine; PC, phosphatidylcholine; PEG-PE, pegylated phosphatidylethanolamine; PG, phosphatidylglycerol; RES, reticuloendothelial system.

those conventional liposomes that have reached clinical trials. The earliest studies sought to define SULV formulations that reduced normal tissue drug deposition but retained equivalent antitumour activity to the unencapsulated drug (Gabizon et al 1982). Subsequent studies went one step further and generated liposomes with both favourable patterns of normal tissue distribution and improved tumour targeting (Gabizon et al 1983; Proffitt et

al 1983). One such neutral SULV composed of a 2:1 ratio of distearoyl phosphatidylcholine (DSPC) and cholesterol has been extensively evaluated because of its high level of tumour targeting and modest levels of RES uptake (Forssen et al 1992, 1996). A systematic evaluation of SULV formulations composed of phospholipid and cholesterol has shown that DSPC in a ratio of 1:1 or 2:1 relative to cholesterol is optimal (Ogihara-Umeda et al 1996). As a consequence of these studies, a DSPC/cholesterol liposomal preparation of daunorubicin has entered clinical studies as DaunoXome (see below). An alternative formulation composed of egg phosphatidylcholine (PC) and cholesterol (ratio 1.2:1) has also been shown to deliver anthracyclines effectively to solid and intraperitoneal tumour xenografts (Harasym et al 1997). This formulation has been taken in to clinical studies as TLC D-99 (see below). In addition to studies with anthracyclines, DSPC/cholesterol liposomes containing vincristine have been developed through pre-clinical studies. Liposome-encapsulated vincristine has been shown to be more active and less toxic than the unencapsulated agent in a number of murine models (Mayer et al 1990; Boman et al 1994; Webb et al 1995). The end result of these studies has been the development of a DSPC/cholesterol liposome formulation of vincristine (ONCO-TCS) that has entered phase I trials (see below).

In contrast to liposomal anthracycline and vinca alkaloid preparations, the development of conventional liposomal platins has involved the use of MLV rather than SULV. MLV composed of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG) (ratio 7:3) containing the lipophilic platin *cis*-bis-neodecanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II) (NDDP) have been shown to yield equivalent or increased tumours levels compared with unencapsulated cisplatin after intravenous administration (Khokhar et al 1988). In addition, this agent appeared to have particularly attractive pharmacokinetics after intraperitoneal injection (Vadiei et al 1992), which has promoted its use as an intracavitary agent in patients with malignant effusions in early clinical trials (see below).

*Sterically stabilized liposomes*

A summary of the published pre-clinical data on the biodistribution and pharmacokinetics of sterically stabilized liposomes is presented in Table 2. As has been detailed above, sterically stabilized liposomes evolved from studies in which various components were added to the membrane and their effect on in-vivo longevity was assessed (Gabizon & Papahadjopoulos 1988; Allen & Hansen 1991; Klibanov et al 1990; Papahadjopoulos et al 1991). Of the three agents, GM1, HPI and MPEG, only the latter has given rise to a family of liposomal agents that have entered clinical usage. Klibanov et al (1990) were the first to show that incorporation of PEG-conjugated phosphatidylethanolamine (PEG-PE) into LULV composed of egg PC and cholesterol (ratio 1:1) increased the blood circulation half-life by more than 10-fold compared with unpegylated liposomes ($t_{1/2}$ = 5 h vs < 30 min). Subsequently, a number of studies have confirmed these data and have demonstrated the ability of liposomes to accumulate in tumour tissues in rodent models (Huang et al 1992a, b, 1993; Vaage et al 1994, 1997; Siegal et al 1995; Newman et al 1999; Harrington et al 2000a, b). Studies using intravenous injections of gold particles entrapped in egg PC/cholesterol liposomes containing GM1 or a PEG-PE derivative have shed light on the microbiodistribution of these agents. On electron microscopy, intracytoplasmic colloidal gold was observed in hepatic Kuppfer cells and bone marrow macrophages, confirming the role of these cells in clearing circulating liposomes. In xenograft solid tumours and Kaposi's sarcoma (KS)-like dermal lesions in transgenic mice, gold particles were seen in blood vessels and in a perivascular cuff beyond the endothelium (Huang et al 1992b, 1993). There was no sign of colloidal gold in the cytoplasm of tumour cells, suggesting that, for pegylated liposomes, endocytosis by tumour cells does not occur to a significant extent (Huang et al 1992b). Uptake was more pronounced in the KS-like lesions than in the adjacent normal skin (Huang et al 1993). More recently, the liposomal formulation has been adjusted to contain hydrogenated soy PC and microfluorometric studies have provided elegant data that show pegylated liposomes accumulating in the extracellular space of tumours, where they release their contents, which are subsequently distributed throughout the tumour (Vaage et al 1994, 1997). Ishida et al (1999) have demonstrated interstitial permeation by sterically stabilized liposomes, the vast majority of which were taken up by tumour-associated macrophages. A recent study has determined the detailed biodistribution and pharmacokinetics of radiolabelled liposomes in tumour and a range of normal tissues in mice (Harrington et al 2000a). As a direct consequence of these studies, pegylated liposomes incorporating methoxypolyethylene glycol-derivatized distearoyl phosphatidylethanolamine (MPEG-DSPE) have been developed for clinical use with doxorubicin (Caelyx/Doxil) and cisplatin (SPI-077), and another formulation containing vincristine is under development (Allen et al 1995).

## Pre-clinical therapeutic studies

*Conventional liposomes*

The main emphasis of development of conventional liposomal agents was initially directed towards the anthracyclines (Table 3). The reasons for this selection are easy to appreciate: (i) they are relatively easy to formulate in liposomes; (ii) they exhibit a broad range of antitumour activity; and (iii) they cause a relatively predictable dose-limiting cardiotoxicity, which provides a powerful model for studying the effect of liposomal encapsulation on the toxicity profile of drugs. This choice appears to have been rather fortunate since these agents have demonstrated efficacy in therapeutic models, whereas other classes of chemotherapeutic drugs have been less successfully developed. The earliest studies demonstrated the ability of liposomes containing doxorubicin to reduce cardiac drug localization without reducing the antitumour efficacy of the entrapped agent (Rahman et al 1980; Gabizon et al

**Table 3** Summary of pre-clinical therapeutic studies of conventional liposomal anthracyclines in animal models.

| Liposomes | Agent | Tumour models | Outcome | Reference |
|---|---|---|---|---|
| PS/PC/Chol<br>SA/PC/Chol | DOX | Lewis lung cancer<br>P388 ascitic leukaemia | A comparison was performed between positively and negatively charged liposomes. Positively charged (SA/PC/Chol) liposomes had equivalent therapeutic activity, but reduced cardiotoxicity, compared with unencapsulated drug | Rahman et al 1980 |
| PS/PC/Chol<br>PC/Chol<br>DPG/PC/Chol | DOX | J-6456 (lymphoma) | There was significantly increased survival for animals treated with PS/PC/Chol liposomal doxorubicin compared with the unencapsulated drug. Survival after PC/Chol and DPG/PC/Chol liposomal doxorubicin was equivalent to that seen with the unencapsulated drug | Gabizon et al (1982) |
| PS/PC/Chol | DOX | Lewis lung cancer<br>Mouse sarcoma 180 | In comparison with unencapsulated doxorubicin, liposomal doxorubicin was significantly more effective against Lewis lung cancer and had equivalent activity against mouse sarcoma 180 | Forssen et al 1983 |
| PS/PC/Chol<br>SA/PC/Chol | DOX | IgM immunocytoma | Negatively charged (PS/PC/Chol) liposomes had equivalent activity and decreased cardiotoxicity compared with unencapsulated doxorubicin. Positively charged (SA/PC/Chol) liposomes had reduced therapeutic effect | van Hoesel et al (1984) |
| PS/PC/Chol<br>PG/PC/Chol | DOX | J-6456 (lymphoma) | There was significantly increased survival with liposomal compared with unencapsulated drug | Gabizon et al (1985) |
| PG/PC/Chol | DOX | None | The rate of drug-related deaths in mice treated with liposomal compared with unencapsulated doxorubicin was significantly reduced. There was reduced cardiac, renal, hepatic and biochemical (hyperlipidaemia, hypoglycaemia) toxicity with liposomal doxorubicin | Gabizon et al (1986) |
| PG/PC/Chol/Toc | DOX | CT38LD (colon)<br>CT26 (colon) | The CT38LD cell line was more sensitive than CT26 to doxorubicin in-vitro. Liposomal doxorubicin was significantly more active than the unencapsulated agent when administered according to a protracted dose schedule against CT38LD and CT26 tumours in-vivo | Mayhew et al (1987) |
| DSPC/Chol | DNR | P-1798 (lymphosarcoma)<br>MA16C (breast) | Liposomal DNR was more active than the unencapsulated agent against both P-1798 and MA16C models. At the MTD, there were 100% cures for liposomal DNR compared with 40% for unencapsulated DNR against MA16C. In the P-1798 model, the liposomal agent was only more effective than the unencapsulated drug at lower doses | Forssen et al (1992) |

Chol, cholesterol; DNR, daunorubicin; DOX, doxorubicin; DPG, diphosphatidylglycerol; DSPC, distearoyl phosphatidylcholine; MTD, maximum tolerated dose; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; SA, stearylamine; Toc, α-tocopherol.

1982; van Hoesel et al 1984). Further studies identified liposomal preparations that enhanced the therapeutic effect relative to the unencapsulated agent by increasing drug delivery within tumour cells. This effect was achieved at the same time as reducing treatment-related toxicity (Forssen & Tokes 1983; Gabizon et al 1985; Mayhew et al 1987).

Tabl 4. Summary of pre-clinical therapeutic studies of conventional liposomal platinum analogues in animal models.

| Liposomes | Agents | Tumour models | Outcome | Reference |
|---|---|---|---|---|
| DMPC/DMPG (L-NDDP) | NDDP CDDP | L1210 (leukaemia) M5076 (reticulosarcoma) | L-NDDP was more active than unencapsulated NDDP or CDDP administered by the intraperitoneal route against intraperitoneal L1210 tumours. L-NDDP administered by the intraperitoneal route retained its activity against platin-resistant intraperitoneal L1210 tumours. L-NDDP was more active than unencapsulated CDDP when administered intravenously against the M5076 liver metastasis model | Perez-Soler et al (1987) |
| PS/PC/Chol (L-CDDP1) DPPC/DPPG/Chol (L-CDDP2) | CDDP | IgM immunocytoma | Both L-CDDP1 and L-CDDP2 were less effective than unencapsulated CDDP. Increased platinum levels were documented in renal tissue with L-CDDP1, but this was associated with less nephrotoxicity than unencapsulated CDDP | Steerenberg et al (1988) |
| DMPC/DMPG (L-NDDP) | NDDP | L1210 (leukaemia) L1210/PDD (leukaemia) M5076 (reticulosarcoma) | L-NDDP was more active than unencapsulated NDDP or CDDP in-vitro. L-NDDP was more active than unencapsulated NDDP administered by the intraperitoneal route against intraperitoneal L1210 and L1210/PDD tumours in-vivo. L-NDDP was more active than unencapsulated NDDP administered by the intravenous route against M5076 liver metastases in-vivo | Perez-Soler et al (1988) |
| DMPC/DMPG (L-NPDP, L-NDDP, L-DEDP) | NPDP NDDP DEDP | L1210 (leukaemia) M5076 (reticulosarcoma) B16 (melanoma) | L-NPDP, L-NDDP and L-DEDP had equivalent activity to unencapsulated CDDP as single injections by the intraperitoneal route against intraperitoneal L1210 tumours. L-NPDP, L-NDDP and L-DEDP were more active than unencapsulated CDDP as multiple intraperitoneal injections against intraperitoneal L1210 tumours. Only intravenous L-NDDP demonstrated any activity against intravenously administered L1210 tumours. L-NDDP and L-DEDP were more effective than unencapsulated CDDP against liver metastases of M5076. L-NPDP, L-NDDP and L-DEDP demonstrated activity against intraperitoneal implanted B16 tumours | Khokhar et al (1989) |

**Table 4** (*cont*).

| Liposome | Agents | Tumour models | Comments | Reference |
|---|---|---|---|---|
| PC/PS/Chol (L-CDDP) | CDDP | L1210 (leukaemia) NIH OVCAR (ovary) | There was no difference between unencapsulated CDDP and L-CDDP in terms of in-vitro cytotoxicity or in-vivo activity after intravenous injection. Significantly increased antitumour activity was demonstrated for L-CDDP compared with unencapsulated CDDP after intraperitoneal injection in-vivo. L-CDDP caused a significantly lower rate of fatal toxicity than unencapsulated CDDP in-vivo | Gondal et al (1993) |

CDDP, *cis*-dichlorodiammine platinum (II); Chol, cholesterol; DEDP, *cis*-bis-*n*-decanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II); DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; DPPC, dipalmitoyl phosphatidylcholine; DPPG, dipalmitoyl phosphatidylglycerol; NDDP, *cis*-bis-neodecanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II); NPDP, *cis*-bis-neopentanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II); PC, phosphatidylcholine; PS, phosphatidylserine.

For example, in these studies, toxic death, cardiomyopathy, renal, hepatic and biochemical (hyperlipidaemias and hypoglycaemia) toxicities were appreciable for the unencapsulated drug but negligible for liposomal doxorubicin. Data such as these provided the background for the development of PC/cholesterol liposomes containing doxorubicin (TLC-D99) for clinical study. The activity of daunorubicin in a liposome composed of DSPC/cholesterol has also been reported (Forssen et al 1992; Forssen & Ross 1994). This agent has been shown to yield superior response rates to those seen with the unencapsulated agent and is undergoing clinical evaluation as DaunoXome.

Two issues have dominated the pre-clinical therapeutic assessment of conventional liposomal platinum compounds: (i) the need to reduce the systemic toxicity of the available platins (cisplatin, carboplatin); and (ii) the desire to encapsulate novel lipophilic platinum analogues that cannot be formulated in the aqueous phase (Table 4). As regards the first of these issues, liposomal forms of cisplatin have been shown to exhibit reduced nephrotoxicity and to have higher maximum tolerated doses than the unencapsulated agent, although this effect may be achieved at the expense of some clinical activity (Steerenberg et al 1988; Gondal et al 1993). As for the second issue, in recent years, the development of novel lipophilic platinum analogues has concentrated on examination of various NDDP complexes (Perez-Soler et al 1987, 1988; Khokhar et al 1989). Under these circumstances, rapid drug leakage from the liposome after administration may not be seen as a major problem since the issue is really one of ease of drug administration. Indeed, the use of liposomal encapsulation offers a potential means of administering other agents such as taxanes, which are difficult to formulate (Cabanes et al 1998a). A number of lipophilic platins encapsulated in liposomes composed of DMPC and DMPG (in a 7:3 molar ratio) have been shown to exhibit activity against tumour models in-vivo. NDDP appears to be most promising, with either equivalent or superior antitumour activity to cisplatin, but significantly reduced nephrotoxicity. This agent has entered clinical studies as both an intravenous and intraperitoneal/intrapleural therapeutic agent.

*Sterically stabilized liposomes*

As with the conventional liposomes, most of the published data on the therapeutic efficacy of sterically stabilized liposomal agents deal with anthracyclines (Table 5). The formulation that has become Caelyx/Doxil has been shown to exert significant activity against a broad range of syngeneic and xenograft tumours in rodent models. In general, these effects have been shown to exceed those of comparable conventional liposomal formulations, with appreciable amelioration of treatment-related toxicity (Gabizon 1992; Huang et al 1992a; Vaagé et al 1992, 1993a, b, 1994, 1995, 1997; Williams et al 1993; Siegal et al 1995; Cabanes et al 1998b; Harrington et al 2000c).

In addition, the therapeutic efficacy of a number of non-anthracycline compounds encapsulated in sterically stabilized liposomes has been assessed (Table 6). Studies have been performed with vincristine (Allen et al 1995; Vaage et al 1993a), mitoxantrone (Chang et al 1997) and platinum analogues (Mori et al 1996; Colbern et al 1999; Newman et al 1999; Vaage et al 1999; Harrington et al 2000c). In the case of vincristine and mitoxantrone, liposomal encapsulation was shown to significantly increase their antitumour activity, while, for vincristine, reducing the toxicity of treatment. As yet, neither of these agents has been further developed towards clinical trials. The activity of pegylated liposome encapsulated cisplatin (SPI-077) has also been

**Table 5** Summary of pre-clinical therapeutic studies of sterically stabilized liposomal anthracyclines in animal models.

| Liposomes | Agents | Tumour models | Comments | Reference |
|---|---|---|---|---|
| HPC/Chol/HPI (PL-DOX, PL-EPI) PC/PG/Chol (L-DOX, L-EPI) | DOX EPI | J-6456 (lymphoma) | PL-DOX was significantly more effective than L-DOX or unencapsulated DOX. L-DOX, in turn, was significantly more effective than unencapsulated DOX | Gabizon (1992) |
| PC/PG/Chol/BHT (L-DOX) HSPC/Chol/PEG-DSPE/Toc (PL-DOX) | DOX | MC19/MC65/MC2A/ MC2B (breast) | Therapy was associated with reduced metastasis from intramammary tumour implants and increased cure rate of subcutaneous implants. PL-DOX was significantly better than L-DOX or unencapsulated DOX | Vaage et al (1992) |
| HSPC/Chol (L-DOX, L-EPI) HSPC/Chol/PEG-DSPE/Toc (PL-DOX, PL-EPI) | DOX EPI | C-26 (colon) | PL-DOX and PL-EPI were significantly more effective than L-DOX or L-EPI | Huang et al (1992a) |
| PC/PG/Chol/Toc (L-EPI) HSPC/Chol/PEG-DSPE/Toc (PL-EPI) | EPI | C26 (colon) | PL-EPI was significantly more active than either L-EPI or unencapsulated EPI | Mayhew et al (1992) |
| HSPC/Chol/PEG-DSPE (PL-DOX) | DOX | MC2 (breast) | PL-DOX was significantly more effective than unencapsulated DOX | Vaage et al (1993a) |
| HSPC/Chol/PEG-DSPE (PL-DOX) | DOX | HEY (ovary) | PL-DOX was significantly more effective than unencapsulated DOX by both intravenous and intraperitoneal routes. There was a significant reduction in toxicity associated with the intraperitoneal route for PL-DOX | Vaage et al (1993b) |
| PC/PG/Chol/Toc (L-DOX) HSPC/Chol/PEG-DSPE/Toc (PL-DOX) | DOX | TL-1 (lung) | PL-DOX was significantly more active than either L-DOX or unencapsulated DOX | Williams et al (1993) |
| HSPC/Chol/PEG-DSPE (PL-DOX) | DOX | PC-3 (prostate) | PL-DOX was significantly more active than unencapsulated DOX | Vaage et al (1994) |
| HSPC/Chol/PEG-DSPE (PL-DOX) | DOX | Malignant fibrous histiocytoma (sarcoma) | Treatment with PL-DOX significantly increased the lifespan of animals compared with unencapsulated DOX | Siegal et al (1995) |
| HSPC/Chol/PEG-DSPE (PL-DOX) | DOX | C3H/He mammary cancer (breast) | PL-DOX significantly reduced pulmonary metastases and increased survival compared with placebo | Vaage et al (1995) |
| HSPC/Chol/PEG-DSPE (PL-DOX) | DOX | AsPC-1 (pancreas) | PL-DOX was significantly more active than unencapsulated DOX | Vaage et al (1997) |

Table 5 (*cont*).

| Liposomes | Agents | Tumour models | Comments | Reference |
|---|---|---|---|---|
| HSPC/Chol/PEG-DSPE (PL-DOX) | DOX | J6456 (ascitic lymphoma) | PL-DOX significantly more active than unencapsulated DOX. For PL-DOX, the intravenous route was more active than the intraperitoneal route. In contrast, for DOX, the intraperitoneal route was more active than the intravenous route | Cabanes et al (1998b) |
| HSPC/Chol/PEG-DSPE (PL-DOX) | DOX | KB (head and neck cancer) | Unencapsulated DOX was significantly more active than PL-DOX in-vitro (IC50 12-fold lower for unencapsulated DOX). PL-DOX was significantly more active and better tolerated than unencapsulated DOX in-vivo | Harrington et al (2000c) |

DOX, doxorubicin; EPI, epirubicin; PC, phosphatidylcholine; PG, phosphatidylglycerol; Chol, cholesterol; BHT, butylated hydroxytoluene; HSPC, hydrogenated soy phosphatidylcholine; PEG-DSPE, PEG-derivatized distearoyl phosphatidylethanolamine; Toc, $\alpha$-tocopherol; PEG-DPPE, PEG-derivatized dipalmitoyl phosphatidylethanolamine; IC50, drug concentration that caused 50% cell survival.

reported in a number of studies. This agent has been shown to be more active than unencapsulated cisplatin, with a greatly reduced toxicity profile (Colbern et al 1999; Newman et al 1999; Vaage et al 1999; Harrington et al 2000c). However, the degree of enhancement of activity of the parent compound appeared to be less than that reported for pegylated liposomal doxorubicin (Harrington et al 2000c).

## Clinical biodistribution and pharmacokinetic studies

*Conventional liposomes*

There have been a limited number of clinical biodistribution and pharmacokinetic studies in liposomes that have entered clinical practice and they are summarized in Table 7. Imaging studies using radiolabelled liposomes composed of DSPC and cholesterol have confirmed the ability of this formulation to target a range of solid and haematological tumours (Presant et al 1988, 1990; Turner et al 1988; Gabizon et al 1991; Kubo et al 1993; Khalifa et al 1997). Kubo et al (1993) injected $^{111}$In-nitrilotriacetic acid (NTA)-labelled liposomes containing 37 MBq of radioactivity into seven patients with breast (n = 2), colonic (n = 2), lung (n = 1) and prostate cancers (n = 1), and lymphoma (n = 1). Pharmacokinetic analysis revealed 23±5% of the injected dose per litre of blood (% ID $L^{-1}$) at 3 min, and 8.9±7.5% ID $L^{-1}$ at 4 h. P sitive tumour images were obtained in four patients (1/2 breast, 2/2 colonic, 0/1 lung, 1/1 prostate and 0/1 lymphoma). Prominent hepatic uptake was demonstrated with 32.6±14.4% of the injected dose and 34.8±8.4% of the injected dose in that organ at 4 and 48 h, respectively. Khalifa et al (1997) have reported the ability of identical $^{111}$In-NTA-labelled liposomes to localize to recurrent high-grade gliomas in eight patients. The tumours were seen clearly in seven of the eight patients. The total uptake of the radiolabelled liposomes in the brain was estimated at approximately 1.1%, with a maximum tumour to normal tissue ratio of 1.4:1. Significant hepatic uptake was observed at up to 50% of the injected dose.

*Sterically stabilized liposomes*

The only sterically stabilized liposomes that have undergone clinical evaluation are those that contain MPEG-derivatized lipids in their membranes. Therefore, this discussion of localization studies of sterically stabilized liposomes will be restricted to studies in which pegylated liposomes have been used. Gabizon et al (1994) assessed the pharmacokinetics of doxorubicin in seven patients after injections of equivalent doses of unencapsulated and/or pegylated liposomal drug, and in a further nine patients after administration of the liposomal drug alone. Plasma elimination of pegylated liposomal doxorubicin was shown to follow a biexponential curve with median $t_{1/2}\alpha$ and $t_{1/2}\beta$ of 2 and 45 h, respectively. Almost 100% of the drug detected in the plasma was in the liposomal form. The plasma clearance of pegylated liposomal doxorubicin was significantly lower than for the unencapsulated agent (0.1 L $h^{-1}$ vs 45 L $h^{-1}$), as was the volume of distribution (4 L vs 254 L). The drug levels achieved in malignant

**Table 6** Summary of pre-clinical therapeutic studies of non-anthracycline drugs encapsulated in sterically stabilized liposomes.

| Liposomes | Agents | Tumour models | Outcome | Reference |
|---|---|---|---|---|
| HSPC/Chol/MPEG-DSPE (PL-VCR and PL-DOX) | VCR DOX | MC2 (breast) | PL-VCR was more effective than unencapsulated VCR. Combination therapy of PL-VCR and PL-DOX given simultaneously was less effective than either agent given as a single dose. Combination therapy of PL-VCR and PL-DOX given according to an alternating schedule was more effective than single dose treatment with either agent | Vaage et al (1993a) |
| PC/Chol<br>PC/Chol/GM1<br>PC/Chol/PEG-PE<br>DMPG/DMPC | NDDP | RIF-1 (sarcoma) | PEG-PE liposomal NDDP exhibited significantly greater cytotoxicity in-vitro than GM1 liposomal NDDP. PEG-PE liposomal NDDP was significantly more effective in-vivo than PC/Chol, DMPC/DMPG and GM1-liposomal NDDP | Mori et al (1996) |
| DSPC/Chol (L-MITO)<br>DSPC/Chol/PEG-DPPE (PL-MITO) | MITO | intravenous L1210 (leukaemia) | L-MITO and PL-MITO were significantly less toxic than unencapsulated MITO. L-MITO was as active as PL-MITO and each was more effective than unencapsulated MITO only at highest dose level | Chang et al (1997) |
| HSPC/Chol/MPEG-DSPE (PL-CDDP) | CDDP | HT29 (colon) | PL-CDDP was significantly more effective than unencapsulated CDDP | Vaage et al (1999) |
| HSPC/Chol/MPEG-DSPE (PL-CDDP) | CDDP | Lewis lung tumour C26 (colon) | PL-CDDP was significantly more effective than unencapsulated CDDP. Equivalent levels of tumour control were achieved with a 50% dose reduction of PL-CDDP | Newman et al (1999) |
| HSPC/Chol/MPEG-DSPE (PL-CDDP) | CDDP | BT474 (breast)<br>MDA453 (breast) | Unencapsulated CDDP and PL-CDDP were both effective as single agents against xenograft tumours. At tolerable dose levels, PL-CDDP was superior to unencapsulated CDDP. Both agents enhanced the activity of a humanized monoclonal antibody directed against HER2 (Herceptin) | Colbern et al (1999) |

Table 6 (*cont*).

| Liposomes | Agents | Tumour models | Comments | Reference |
|---|---|---|---|---|
| HSPC/Chol/MPEG-DSPE (PL-CDDP) | CDDP | KB (head and neck) | Unencapsulated CDDP was significantly more active than PL-CDDP in-vitro (IC50 21-fold lower for CDDP). PL-CDDP displayed superior activity to unencapsulated CDDP in-vivo, but only at the intermediate dose level. Toxicity was significantly reduced for PL-DOX | Harrington et al (2000c) |

CDDP, *cis*-dichlorodiammine platinum (II); Chol, cholesterol; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; DOX, doxorubicin; DSPC, distearoyl phosphatidylcholine; GM1, monosialoganglioside; HSPC, hydrogenated soy phosphatidylcholine; MITO, mitoxantrone; MPEG-DSPE, PEG-derivatized distearoyl phosphatidylethanolamine; NDDP, *cis*-bis-neodecanoato *trans-R,R*-1,2diaminocyclohexane platinum (II); PC, phosphatidylcholine; PEG-DPPE, PEG-derivatized dipalmitoyl phosphatidylethanolamine; VCR, vincristine.

effusions were assessed where possible. The liposomal agent demonstrated a 4- to 6-fold increase compared with the unencapsulated agent, peaking between 3 and 7 days after drug administration. Northfelt et al (1996) confirmed the efficacy of pegylated liposomal doxorubicin in targeting cutaneous AIDS-related KS in a study in which 18 patients were randomly allocated to receive either pegylated liposomal doxorubicin or unencapsulated doxorubicin. Representative lesions were biopsied 72 h after drug administration. The doxorubicin level in the KS lesions was 5.2- to 11.4-times greater in those patients treated with the pegylated liposomal form of the drug. The detailed pharmacokinetic data revealed that the volume of distribution of doxorubicin varied from 2.2 to 4.4 $L\,m^{-2}$, demonstrating that the drug was essentially confined to the circulation. The clearance of pegylated liposomal doxorubicin was 0.034–0.108 $L\,h^{-1}\,m^{-2}$ and the $t_{1/2}\alpha$ and $t_{1/2}\beta$ were 3.8 and 41.3 h, respectively. In a study using $^{111}$In-DTPA-labelled pegylated liposomes of the same formulation as used for Caelyx/Doxil, Harrington et al (2001a) have demonstrated the ability of these liposomes to target locally advanced solid tumours, including breast, lung, head and neck, and cervical cancers.

## Clinical therapeutic studies

*Conventional liposomes*

*Liposomal doxorubicin (TLC D-99, Evacet).* Liposomal doxorubicin (TLC D-99; The Liposome Company, Princeton, New Jersey, USA) consists of doxorubicin entrapped in liposomes composed of egg PC and cholesterol (ratio 1.22:1). This agent has been the subject of pharmacokinetic and phase I, II and III clinical trials (Cowens et al 1993; Embree et al 1993; Batist et al 1998, 1999; Harris et al 1998; Cheung et al 1999; Shapiro et al 1999; Valero et al 1999). The pharmacokinetics of TLC D-99 at doses of 60 mg $m^{-2}$ and 75 mg $m^{-2}$ have been reported in 12 patients with non-small cell lung cancer, with no difference in the $t_{1/2}\alpha$ and $t_{1/2}\beta$ as compared with unencapsulated doxorubicin (Embree et al 1993). In a phase I clinical study, the drug was administered at single doses of 20–90 mg $m^{-2}$ every 3 weeks and as daily doses for 3 days at doses of 20, 25 and 30 mg $m^{-2}$ (Cowens et al 1993). Leukopenia was the dose-limiting toxicity and set the maximum tolerated dose at a level of 90 mg $m^{-2}$ every 3 weeks or 25 mg $m^{-2}$ $day^{-1}$ for 3 days. In contrast to unencapsulated doxorubicin, gastrointestinal and mucosal toxicity were minimal or absent at each dose level, and significant cardiac, hepatic, renal or other organ toxicities were not seen. TLC D-99 has been administered to 40 patients with AIDS-related KS in the setting of a randomized phase II trial of either 10 mg $m^{-2}$ (19 patients) or 20 mg $m^{-2}$ (21 patients) every 2 weeks. Partial responses occurred in only 15% (6 of 40) of patients and a further 65% (26 of 40) showed stable disease. Treatment response was dose-related, with 5% (1 of 19) of patients in the low-dose cohort achieving a partial response in comparison with 24% (5 of 21) of patients in the high-dose group. The major toxicity observed was haematological, with neutropenia occurring in 68% and 81% of patients in the low- and high-dose cohorts, respectively. Non-haematological toxicity was mild and alopecia developed in only 8% of patients (Cheung et al 1999). Preliminary findings of a phase III evaluation of TLC D-99 in patients with metastatic breast cancer have been presented in abstract form. A total of 69 patients were randomly allocated to either TLC D-99 (75 mg $m^{-2}$) or unencapsulated doxorubicin (75 mg $m^{-2}$) every 3 weeks, and achieved response rates of 33% and 29%, respectively. The liposomal formulation caused less toxicity; nausea and vomiting (10% vs 25%), stomatitis/mucositis (9% vs 16%) and fever/infection (6% vs 11%) (Harris et al 1998). A separate analysis of this phase III study reported a significant difference in the incidence of reduced left

**Table 7.** Summary of clinical studies assessing tumour localization of conventional and pegylated liposomes in patients with cancer.

| Liposome | Tracer | Patients | Comments | Reference |
|---|---|---|---|---|
| Conventional Egg PC/Chol/PA | $^{99m}TcO_4^-$ | 14 | Patients with various tumours (4 choriocarcinoma, 3 hepatoma, 1 breast, 1 lung, 1 renal cancer, 1 haemangiopericytoma, 1 KS, 1 AML, 1 PRV). Rapid clearance of radiolabelled liposomes by the RES with initial and terminal half-lives of 3 and 8–12 h, respectively. Tumour visualized in only one of 14 patients by gamma camera imaging | Richardson et al (1979) |
| Conventional DSPC/Chol | $^{111}$In-NTA | 24 | Patients with various tumours (5 breast, 6 lung, 3 prostate, 2 colorectal, 1 renal, 1 cervical, 1 thyroid, 1 pancreatic, 1 ovarian cancer, 1 lymphoma, 1 sarcoma, 1 melanoma). Rapid clearance of liposomes with blood levels of 50% and 20% of the injected dose at 4 and 24 h, respectively. Significant uptake of liposomes by the RES. Tumours seen on gamma camera scans at 24 and 48 h in 22 of 24 patients | Turner et al (1988) |
| Conventional DSPC/Chol | $^{111}$In-NTA | 2 | Report of positive tumour images by gamma camera scan in two patients with AIDS-related KS and NHL. No dosimetric data were presented | Presant et al (1988) |
| Conventional DSPC/Chol | $^{111}$In-NTA | 24 | Same group of patients as Turner et al (1988). Additional data presented on two patients who underwent surgery after injection of liposomes. Tumour to blood ratios ranged between 6.5 and 18.3:1 for primary tumour, nodal and liver metastases | Presant et al (1990) |
| Conventional Egg PC/egg PG/Chol | $^{111}$In-deferoxamine | 9 | Rapid clearance of radiolabelled liposomes by the RES. In-vivo evidence of drug leakage from the liposome in the circulation. Only 1/9 tumours demonstrated weakly on gamma camera scan | Gabizon et al (1991) |
| Conventional DSPC/Chol | $^{111}$In-NTA | 7 | Patients with advanced tumours (2 breast, 2 colonic, 1 lung, 1 prostate cancer, 1 lymphoma). Rapid clearance of liposomes to a blood level of 45% of injected dose at 4 h with significant RES uptake. Tumours seen on gamma camera scans at 24 and 48 h in four of seven patients | Kubo et al (1993) |

**Table 7** (*cont.*).

| Liposome | Tracer | Patients | Comments | Reference |
|---|---|---|---|---|
| Pegylated HSPC/Chol/PEG-DSPE | DOX | 16 | Significant prolongation of the half-life, reduction of plasma clearance (0.1 vs 45 L $h^{-1}$) and volume of distribution (4 vs 254 L) of pegylated liposomal doxorubicin compared with unencapsulated drug. Levels of drug 4- to 16-fold greater in malignant effusions in patients treated with liposomal agent | Gabizon et al (1994) |
| Pegylated HSPC/Chol/PEG-DSPE | DOX | 18 | Biopsies of AIDS-related KS lesions after either pegylated liposomal or unencapsulated drug. There was a 5.2 to 11.4-fold increase in tumour drug levels after pegylated liposomal doxorubicin as compared with unencapsulated doxorubicin | Northfelt et al (1996) |
| Conventional DSPC/Chol | 111In-NTA | 8 | Patients with recurrent high grade gliomas. Tumours seen in seven of eight patients on gamma camera scans at 72 h. Total tumour uptake 1% of the injected dose (maximal tumour to normal brain tissue ratio 1.4:1). Significant uptake by the RES (mainly liver and spleen) | Khalifa et al (1997) |
| Conventional DSPC/Chol | DNR | 8 | Patients with recurrent high grade gliomas. Tumour biopsies obtained 24–48 h after DNR administration. Circulation half-life of 4.8 h. Significant tumour accumulation of DNR in the range of anticipated cytotoxic activity | Zucchetti et al (1999) |
| Pegylated HSPC/Chol/PEG-DSPE | DOX | 2 | Patients with metastatic breast cancer who underwent surgical fixation of femoral fracture 6 and 12 days after drug administration. The drug concentration was 10-fold greater in tumour than adjacent normal skeletal muscle | Symon et al (1999) |
| Pegylated HSPC/Chol/PEG-DSPE | $^{99m}TcO_4^-$ | 30 | Patients with NSCLC and SCCHN received radiolabelled pegylated liposomes. Mean tumour to large blood vessel ratio of radioactivity was $1.01 \pm 0.29$ for NSCLC and $1.35 \pm 0.39$ for SCCHN. Liposome uptake correlated with tumour response to liposomal doxorubicin | Koukourakis et al (1999) |

Table 7 (*cont*).

| Liposome | Tracer | Patients | Comments | Reference |
|---|---|---|---|---|
| Pegylated HSPC/Chol/PEG-DSPE | $^{111}$In-DTPA | 20 | Patients with locally advanced cancers (7 SCCHN, 4 NSCLC, 5 breast, 1 cervix cancer, 2 glioma, 1 KS). Tumours seen on gamma camera imaging in 15 of 17 patients studied. Uptake in SCCHN 33.0±5.7% ID kg$^{-1}$, NSCLC 18.3±5.7% ID kg$^{-1}$, breast 5.3±2.7% ID kg$^{-1}$. Tumour uptake related to tumour size. Surgical study in two patients with SCCHN showed tumour to normal tissue ratios of 2- to 11-fold for a range of tissues | Harrington et al (2001a) |

AML, acute myeloblastic leukaemia; Chol, cholesterol; DNR, daunorubicin; DOX, doxorubicin; DSPC, distearoyl phosphatidylcholine; ID, injected dose; KS, Kaposi's sarcoma; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; NTA, nitrilotriacetic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PEG-DSPE, pegylated distearoyl phosphatidylethanolamine; PG, phosphatidylglycerine; PRV, polycythaemia rubra vera; RES, reticuloendothelial system; SCCHN, squamous cell carcinoma of the head and neck.

ventricular ejection fraction between the two groups (16% vs. 25%) in favour of TLC D-99 (Batist et al 1998). These findings have been further supported by more recent data demonstrating a significantly lower rate of cardiotoxicity for TLC D-99 compared with unencapsulated doxorubicin in patients with treatment-naïve breast cancer (Winer et al 2000). In a further phase II study in which the aim was to escalate the dose of TLC D-99 by co-administration of growth factors, 52 patients with treatment-naïve metastatic disease breast received a 135 mg m$^{-2}$ intravenous bolus of TLC D-99 along with 5 $\mu$g kg$^{-1}$ granulocyte colony-stimulating factor via subcutaneous injection every 21 days. The overall response rate was 46%, but this was achieved at the expense of considerable treatment-related toxicity (grade 4 neutropenia, thrombocytopenia, and mucositis in 92%, 88%, and 19% of patients, respectively) (Shapiro et al 1999). Attempts have also been made to incorporate TLC D-99 into combination chemotherapy regimens in patients with metastatic breast cancer (Batist et al 1999; Valero et al 1999). Valero et al (1999) treated 41 patients with TLC D-99 (60 mg m$^{-2}$), cyclophosphamide (500 mg m$^{-2}$) on Day 1, and 5-fluorouracil (500 mg m$^{-2}$) on Days 1 and 8 every 3 weeks. A median of 10 cycles per patient was delivered to a cumulative TLC D-99 dose of 528 mg m$^{-2}$. The regimen was highly active, with an overall response rate of 73%, a median duration of response of 11.2 months and median overall survival of 19.4 months. A phase III study has been presented in abstract form in which patients received cyclophosphamide (600 mg m$^{-2}$) plus 60 mg m$^{-2}$ of either TLC D-99 or doxorubicin (Batist et al 1999). The response rates were identical (43%) for the two regimens, but the liposomal agent was associated with significantly lower rates of myelosuppression and cardiotoxicity.

*Liposomal daunorubicin (DaunoXome).* Liposomal daunorubicin (DaunoXome, NeXstar Pharmaceuticals Inc., San Dimas, USA) is an SULV of 50–80 nm diameter, which consists of phospholipid membranes composed of DSPC and cholesterol in a molar ratio of 2:1. In a phase I/II pharmacokinetic and clinical analysis, this agent demonstrated a favourable alteration of the pharmacokinetic profile of the drug when compared with published data for the unencapsulated drug (Gill et al 1995). At the 80 mg m$^{-2}$ dose level, the mean total body clearances were 6.6 and 233 mL min$^{-1}$ for liposomal and unencapsulated daunorubicin, respectively, which translated to a 36-fold increase in the area under the concentration–time curve. The volume of distribution was 2.9 L for liposomal daunorubicin and 1055 L for unencapsulated daunorubicin. In the early stages of clinical development of this agent, a number of phase II studies were conducted at doses ranging from 40 to 60 mg m$^{-2}$ every 2 weeks in patients with AIDS-related KS. Response rates in the order of 40–73% (Money-Kyrle et al 1993; Presant et al 1993; Gill et al 1995; Girard et al 1996; Uthayakumar et al 1996) were documented. In addition, a large prospective multicentre phase III study of 2-weekly liposomal daunorubicin (40 mg m$^{-2}$) compared with standard combination chemotherapy (ABV: 10 mg m$^{-2}$ doxorubicin, 15 IU bleomycin, 1 mg vincristine) has been completed in 232 patients with AIDS-related KS (Gill et al 1996). The response rates for the two treatment arms were equivalent (25% and 28%, respectiv ly). The toxicity profiles were different, with liposomal daunorubicin causing significantly less neuropathy (13% vs 41%) and alopecia (8% vs 36%) than ABV, but more myelosuppression (grade 3 neutropenia: 36% vs 36%; grade 4 neutropenia: 15% vs 5%). There was a statistically insig-

nificant increase in the incidence of opportunistic infections among the patients treated with liposomal daunorubicin (36% vs 26%).

Thus far, there have been few studies of liposomal daunorubicin in patients with other tumour types. A phase I study of three daily doses of 75–200 mg $m^{-2}$ of liposomal daunorubicin has been conducted in 24 patients with refractory or relapsed acute myeloid leukaemia or blast phase chronic myeloid leukaemia (Cortes et al 1999). The dose-limiting toxicities were myelosuppression, requiring transfusional support and mucositis. There were two complete responses to treatment. In a follow-up study, 62 patients with refractory or recurring acute myeloblastic leukaemia received escalating doses of liposomal daunorubicin at 75, 100, 125, or 135 mg $m^{-2}$ $day^{-1}$ for 3 days with cytosine arabinoside (1 g $m^{-2}$) as a continuous intravenous therapy for 4 days. Eighteen patients (29%) achieved a complete remission, and seven (11%) a haematological improvement for an overall response rate of 40% (Cortes et al 2001). Lippens (1999) treated 14 children with recurrent or progressive brain tumours with a dose of 60 mg $m^{-2}$ once every 4 weeks, up to a cumulative dose of 600 mg $m^{-2}$. There were two complete and three partial responses, and two patients had stable disease. Steele et al (2001) conducted a phase I/II trial in 14 patients with pleural malignant mesothelioma. The maximum tolerated dose was 120 mg $m^{-2}$ every 3 weeks. No objective responses to treatment were recorded, although nine patients had stabilization of their disease during therapy. Two separate phase I and II studies of liposomal daunorubicin have been reported in patients with hepatocellular carcinoma (Yeo et al 1999; Daniele et al 2000). Yeo et al (1999) treated 14 patients with doses of 100 mg $m^{-2}$ every 3 weeks, but observed no tumour responses. In a phase I trial, 11 patients with hepatocellular carcinoma complicating cirrhosis were enrolled on to a proposed dose-escalation protocol starting at 80 mg $m^{-2}$. Dose-limiting toxicity was encountered at the starting dose and at the subsequent reduced doses of 60 mg $m^{-2}$ and 40 mg $m^{-2}$ (Daniele et al 2000). More recently, a phase I dose-escalating trial of DaunoXome was conducted in 16 patients with metastatic breast cancer (O'Byrne et al 2002). Of 15 evaluable patients, two had partial responses, six had stable disease and seven progressed. The dose-limiting toxicity was neutropenic fever at a dose of 120 mg $m^{-2}$.

*Liposomal vincristine (Onco-TCS).* This formulation of vincristine entrapped in 120-nm SULV composed of DSPC and cholesterol has undergone preliminary clinical investigation (Gelmon et al 1999; Sarris et al 2000). Gelmon et al (1999) treated 25 patients with various malignancies with doses of liposomal vincristine between 0.5 and 2.8 mg $m^{-2}$ every 3 weeks. Toxicity was pain, constipation, fever, rigors, fatigue, myalgia and peripheral neuropathy and the maximum dose was 2.4 mg $m^{-2}$. A single partial response was seen in a patient with pancreatic cancer and minor responses were seen in two patients. Sarris et al (2000) reported the results of a phase II trial in 51 patients with relapsed non-Hodgkin's lymphomas and acute lymphoblastic leukaemia. Patients received 2.0 mg $m^{-2}$ every 2 weeks and responses were seen in 14 of 34 (41%) evaluable patients. Severe (grade 3 or 4) motor or sensory neuropathy was seen in 11 patients and necessitated termination of therapy in five patients (all of whom had prior neuropathy).

*Liposomal NDDP.* NDDP encapsulated in conventional MLV has been subjected to a number of phase I/II studies. A dose-escalation phase I study of intravenous liposomal NDDP administered every 4 weeks has been conducted in 39 patients with a range of tumour types (Perez-Soler et al 1990). The dose-limiting toxicity was myelosuppression and the maximum tolerated dose was 312.5 mg $m^{-2}$ every 4 weeks. Other significant toxicities included nausea, vomiting and diarrhoea, but there was no evidence of nephrotoxicity or ototoxicity. The favourable pharmacokinetics documented after intraperitoneal administration in mice (Vadiei et al 1992) have prompted studies of locoregional administration of this agent in patients with malignant effusions (Perez-Soler et al 1997, 1999). In the first study, 21 patients with non-loculated malignant pleural effusions due to lung cancer, ovarian cancer and mesothelioma were treated with escalating doses of intrapleural liposomal NDDP (Perez-Soler et al 1997). The maximal tolerated dose was 450 mg $m^{-2}$ and the limiting toxicity was chest pain, which presumably occurred secondary to chemical pleuritis. Pharmacokinetic analysis demonstrated initial rapid drug absorption into the circulation over 2 h, followed by plateau levels between 6 and 24 h. Intrapleural administration abrogated the toxicities associated with intravenous administration. One patient experienced complete resolution of his malignant effusion and five others had significant volume reductions for prolonged periods. In a follow-up study, 20 patients with mesothelioma received liposomal NDDP at a dose of 450 mg $m^{-2}$ every 3 to 4 weeks (Perez-Soler et al 1999). Two patients died soon after the first drug administration due to local complications of the infusion. In the assessable patients, 11 of 15 (73%) patients had a complete pathological response of their disease.

*Sterically stabilized liposomes*

*Pegylated liposomal doxorubicin (Doxil/Caelyx).* Pegylated liposomal doxorubicin (Doxil/Caelyx; SEQUUS Pharmaceuticals Inc., Menlo Park, USA) consists of doxorubicin encapsulated in an SULV of mean diameter 96 nm with the following lipid composition (values expressed in % molar ratio): hydrogenated soybean phosphatidylcholine (HSPC) (56.2%), cholesterol (38.3%), and *N*-(carbamoyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-3-phospho-ethanolamine sodium salt (5.3%). Initially, as in the case of DaunoXome, this agent was subjected to phase I and II studies in patients with AIDS-related KS. Response rates of 69–93% were reported, with relatively little toxicity (Hengge et al 1993; Simpson et al 1993; Bogner et al 1994; James et al 1994; Harrison et al 1995; Goebel et al 1996). Subsequently, two large phase III studies

were reported in which single agent Doxil/Caelyx was randomized against standard combination chemotherapy for AIDS-related KS; either bleomycin and vincristine (BV) (Stewart et al 1998) or ABV (Northfelt et al 1998). In the former study, 241 patients were treated with either Doxil/Caelyx (20 mg $m^{-2}$) or bleomycin (15 IU $m^{-2}$) and vincristine (2 mg) every 3 weeks for 6 cycles (Stewart et al 1998). Doxil/Caelyx was significantly more active than BV, with response rates of 58.7% and 23.3%, respectively. Patients treated with BV were significantly more likely to stop treatment prematurely because of an adverse event (26.7% vs 10.7%) and had greater rates of peripheral neuropathy (14.2% vs 3.3%) and lower rates of leukopenia (50.8% vs 71.9%). In the latter study, 258 patients were randomized to receive either Doxil/Caelyx (20 mg $m^{-2}$) or doxorubicin (20 mg $m^{-2}$), bleomycin (10 IU $m^{-2}$) and vincristine (1 mg) every 2 weeks for 6 cycles (Northfelt et al 1998). Doxil/Caelyx was significantly more effective than ABV, with response rates of 45.9% and 24.8%, respectively. Doxil/Caelyx was better tolerated than ABV in terms of alopecia (1% vs 19%), nausea and vomiting (15% vs 34%) and neuropathy (6% vs 14%), although there was no difference in grade 3 leukopenia (36% vs 42%). These data are particularly striking in view of the limited activity of single agent unencapsulated anthracycline (and anthracenedione) chemotherapy against AIDS-related KS (Kaplan & Volberding 1985; Chachoua et al 1987; Shepherd et al 1991; Fischl et al 1993).

More recently, a number of phase I/II studies have been conducted in patients with solid cancers. The activity of this agent against refractory ovarian cancer has been reported by a number of groups (Muggia et al 1997; Gordon et al 2000; Israel et al 2000; Markman et al 2000; Johnston and Gore 2001; Muggia and Hamilton 2001). Muggia et al (1997) treated 35 patients with platinum and taxane-resistant ovarian cancer with Doxil/Caelyx at a dose of 40–50 mg $m^{-2}$ every 3–4 weeks. A response rate of 26% (9 of 35) was reported, with one complete and eight partial responses. Drug-induced nausea, alopecia and cardiomyopathy were not seen, although 37% (13 of 35) of patients experienced grade 3 or 4 mucocutaneous toxicity (see below for a discussion of these toxic effects). In a larger study, 89 patients with platinum and taxane-resistant disease received 50 mg $m^{-2}$ Doxil/Caelyx every 4 weeks, with a response rate of 17% (one complete and 14 partial responses). Again, mucocutaneous toxicity was problematic, but other toxicities were uncommon (Gordon et al 2000). In an attempt to develop a more tolerable regimen, Markman et al (2000) treated 49 patients with treatment-refractory ovarian, fallopian tube or peritoneal carcinoma with 40 mg $m^{-2}$ Doxil/Caelyx every 4 weeks. In this case, there were reduced rates of mucocutaneous toxicity, but the response rates were also lower at 9% (4 of 44) of evaluable patients. In a further study, 63 patients with gynaecological cancers, the majority of whom had treatment-refractory ovarian cancer, received 50 mg $m^{-2}$ Doxil/Caelyx every 4 weeks (Israel et al 2000). In patients with measurable disease, the response rate was 19%. Furthermore, in those patients with elevated tumour marker (CA-125) levels, the response rate was 59%. Again, the same patterns of predominantly mucocutaneous toxicity were documented.

Activity of Doxil/Caelyx has also been reported in patients with breast cancer (Ranson et al 1997; Lyass et al 2000; Schwonzen et al 2000). Ranson et al (1997) reported the effect of Doxil/Caelyx in 71 anthracycline-naïve women with stage IV breast cancer. In a rather complex study design, patients received 45–60 mg $m^{-2}$ every 3 to 4 weeks to a maximum of 6 cycles. The overall response rate was 31% and the same number of patients had disease stabilization on treatment. It was determined that a dose of 45 mg $m^{-2}$ every 4 weeks was well tolerated and that dose-limiting cutaneous toxicity precluded the delivery of more dose-intense regimens. In a similar dose-finding and therapeutic study, Lyass et al (2000) treated 45 patients with metastatic breast cancer who had all received chemotherapy. Six separate dose schedules were evaluated ranging from 35 mg $m^{-2}$ every 3 weeks to 70 mg $m^{-2}$ every 6 weeks. Pharmacokinetic parameters were measured in representative patients from each dose schedule and toxicity was found to be dose and schedule dependent. Objective responses were seen in 20% (9 of 45) of patients, with another 20 patients showing either minor response or disease stabilization. More recent studies have focussed on the use of liposomal doxorubicin with radiotherapy and/or hyperthermia in patients with local recurrence (Dvorak et al 2001; Kouloulias et al 2002).

Doxil/Caelyx has also been evaluated in patients with recurrent or metastatic head and neck cancer (Caponigro et al 2000). Twenty four patients were treated at escalating drug doses between 30 mg $m^{-2}$ and 50 mg $m^{-2}$ every 3 weeks. As with the other studies reported, dose-limiting toxicity was mucocutaneous and the maximum tolerated dose was 45 mg $m^{-2}$. The response rate was 33% (8 of 24). Significant activity of this drug was also reported in patients with newly diagnosed, treatment-naïve head and neck cancer (Harrington et al 2001b). Twenty patients were treated, with 10 receiving 2 cycles of 40 mg $m^{-2}$ every 3 weeks before starting radical radiotherapy, and subsequent patients receiving a third escalating dose of 10, 15 or 20 mg $m^{-2}$ 3 days before starting radiotherapy. Nine of 18 (50%) evaluable patients responded to treatment. Significantly, there were no drug-related delays to the delivery of radiotherapy, and local radiation-induced toxicity was not exacerbated. In a different approach, Doxil/Caelyx was administered at escalating doses between 10 mg $m^{-2}$ and 25 mg $m^{-2}$ every 2 weeks concomitant with radiotherapy to patients with head and neck and lung cancers (Koukourakis et al 1999). Treatment was well tolerated, although dose-limiting exacerbation of mucosal toxicity occurred in the patients with head and neck cancer at 20 mg $m^{-2}$.

Wollina et al (2000), in an attempt to exploit the tendency of this agent to localize to the skin, demonstrated significant activity of Doxil/Caelyx in six patients with relapsed cutaneous T-cell lymphoma. Five of six patients responded (83%), with four complete responses and one partial response. In a follow-up report, they presented data for 10 patients. There were six complete and two partial responses, with stable disease in one other patient. Response duration was 15 months and progression-free survival was 18.2

months (Wollina et al 2001). Toma et al (2000) reported a modest response rate of 12% (3 of 25) in patients with advanced pre-treated soft tissue sarcomas who received 30–50 mg $m^{-2}$ every 3 weeks. Significantly, 68% (17 of 25) of the patients achieved temporary stabilization of their disease. O'Brien et al (1998) reported their experience of treating 19 patients with small cell lung cancer with Doxil/Caelyx at a dose of 50 mg $m^{-2}$ every 4 weeks. The majority of patients had been treated previously with platinum-containing combination regimens. In the 10 patients who were evaluable for response, there were three partial responses and a further four patients had disease stabilization.

In contrast to the encouraging data presented above, Doxil/Caelyx has been shown to possess no significant activity against hepatocellular (Halm et al 2000), renal cell (Pennington et al 1998), pancreatic (Yip et al 1999; Halford et al 2001), gastric (Thomas et al 2001) and colorectal (Shields et al 2001) cancers. Trials have also reported poor activity of this drug in patients with advanced soft tissue sarcomas (Elson et al 1998; Skubitz 1998). The preliminary results of a randomized phase II trial of Doxil/Caelyx versus doxorubicin in patients with sarcomas have shown equivalent response rates but significantly less toxicity with the liposomal agent (Judson et al 2001). In addition, preliminary phase I and II studies have been conducted using Doxil/Caelyx in combination with other cytotoxic agents, including paclitaxel (Israel et al 1998; Langley et al 1998; Schwonzen et al 2000; Mavroudis et al 2002), docetaxel (Malik et al 1998), cisplatin (Klein et al 1998) and vinorelbine (Laufman et al 1998; Ramirez et al 1998). A combination regimen of vincristine, Doxil/Caelyx and dexamethasone was shown to exert significant activity in 12 elderly patients with multiple myeloma (Tsiara et al 2000) and offers the potential of avoiding the need for infusional doxorubicin therapy in such patients. Eight patients achieved complete haematological remission and three patients had a partial response. Treatment was given on an ambulatory basis and was well tolerated.

In addition to the potential therapeutic benefits of liposomal formulation, Doxil/Caelyx has been shown to reduce the familiar acute and chronic adverse effects of unencapsulated doxorubicin. Nausea and vomiting is a frequently encountered toxicity of unencapsulated doxorubicin, but is considerably reduced after treatment with Doxil/Caelyx (Harrison et al 1995; Muggia et al 1997; Ranson et al 1997; Stewart et al 1998). Similarly, marked alopecia is almost invariable after prolonged treatment with unencapsulated doxorubicin, but is uncommon (< 10%) in patients treated with Doxil/Caelyx (Harrison et al 1995; Muggia et al 1997; Ranson et al 1997; Northfelt et al 1998; Stewart et al 1998; Gordon et al 2000; Israel et al 2000). Local extravasation of free doxorubicin generally causes severe tissue inflammation and even necrosis (Rudolph et al 1976), but this vesicant activity of the drug is abrogated by encapsulation within a pegylated liposome (Madhavan & Northfelt 1995; Muggia et al 1997; Lotem et al 2000). More importantly, the chronic dose-limiting toxicity of doxorubicin therapy is cardiac damage, resulting in a dilated cardiomyopathy. In general, cumulative life-time doses below a threshold of 550 mg $m^{-2}$ are associated with a low risk of cardiomyopathy, although the incidence rises sharply beyond this dose, effectively imposing a ceiling on the drug dose that may be given safely (von Hoff et al 1979). The incidence of clinically significant doxorubicin-induced cardiotoxicity is considerably reduced by entrapment of the agent within pegylated liposomes, probably because the circulating drug is not bioavailable to the cardiac myocytes, resulting in decreased peak drug concentration and reduced total drug exposure. As a result, total doses of up to 440–840 mg $m^{-2}$ have been tolerated without causing clinically significant cardiac dysfunction. In a recent study, the pathological changes seen on endomyocardial biopsy for a group of 10 patients who had received doses of Doxil/Caelyx ranging from 400 to > 700 mg $m^{-2}$ were compared with a matched control group who had received unencapsulated doxorubicin. The patients who had received the liposomal formulation showed significantly less evidence of damage than those who had received the free drug (Berry et al 1998; Speyer & Wasserheit 1998).

Alteration of the pharmacokinetics and biodistribution of agents by encapsulation within pegylated liposomes has been associated with novel toxic effects. In particular, plantar–palmar erythrodysaesthesia (PPE) or "hand–foot" syndrome is seen in patients treated with repeated doses of Doxil/Caelyx. This toxicity, which manifests as painful swelling and erythema of the hands, feet, intertriginous areas and sites of trauma, is thought to be the result of accumulation of liposomal doxorubicin in the skin, with the effect of delivering a prolonged drug exposure (Gordon et al 1995; Lotem et al 2000). A similar mucosal toxicity associated with aphthous ulceration in the mouth is also seen in association with PPE. Together, these mucocutaneous manifestations represent the main dose-limiting adverse events of Doxil/Caelyx. A study by Vail et al (1998) has assessed the value of pyridoxine in the prevention of PPE in dogs. Dogs with biopsy-confirmed non-Hodgkin's lymphoma were treated with Doxil/Caelyx at a dose of 1 mg $kg^{-1}$ and were randomly allocated to receive either oral pyridoxine (50 mg) 3 times a day or placebo. The relative risk of developing PPE was 4.2-times greater in the dogs treated with placebo. This was associated with pyridoxine-treated dogs receiving a greater dose intensity of Doxil/Caelyx and having a trend towards a better relapse-free survival. In addition, ergotamine administered subcutaneously in rats receiving Doxil/Caelyx has been shown to reduce the incidence of PPE (Colbern et al 1998a). These authors have also conducted a study in mice bearing Lewis lung cancer xenograft tumours, which has demonstrated that the use of prophylactic pyridoxine does not affect the efficacy of Doxil/Caelyx. However, ergotamine was associated with a dose-dependent fall in the therapeutic effect of Doxil/Caelyx. As yet, there have been no published studies of the effect of thes or other agents on the incidence of PPE in patients. Topical 99% dimethylsulfoxide, which has been shown to reduce tissue reactions following extravasation of doxorubicin, has also been shown to have some activity against PPE in a non-randomized study (Lopez et al 1999).

*Pegylated liposomal cisplatin (SPI-077).* Pegylated liposomal cisplatin (SPI-077; SEQUUS Pharmaceuticals Inc.) is a SULV with a mean diameter of 110 nm. It has the following composition (values stated as % molar ratio): 51.0% HSPC, 44.0% cholesterol and 5.0% MPEG-DSPE, and contains 14 $\mu$g cisplatin (mg lipid)$^{-1}$. Pharmacokinetic and preliminary toxicity data for this drug preparation have been reported in abstract form (DeMario et al 1998). Fourteen patients received escalating doses up to 120 mg m$^{-2}$, with no evidence of myelosuppression, neurotoxicity or renal damage. The volume of distribution of the drug was equivalent to plasma volume and the estimated half-life was about 60 h. Analysis of plasma ultrafiltrate revealed no unencapsulated cisplatin, confirming the stability of this liposomal formulation. A dose-escalation study has been performed in patients with non-small cell lung cancer, in which doses of SPI-077 up to 260 mg m$^{-2}$ were administered in combination with paclitaxel, without evidence of dose-limiting platinum-induced toxicity (DeVore et al 1999). A phase I/II study has been conducted in 18 patients with treatment-naïve, locally advanced, inoperable squamous cell carcinoma of the head and neck, who received 2 cycles of treatment at doses of 200–260 mg m$^{-2}$ before commencing radical radiotherapy (Harrington et al 2001c). Treatment was very well tolerated, with no haematological, renal, hepatic or neurological toxicities, but there were partial responses in only two patients (11%). In view of these data and the in-vitro evidence that this liposome has very slow release kinetics, further evaluation of this particular formulation may not be warranted.

## Conclusions

Following a somewhat protracted period of development, liposomally targeted cytotoxic drugs appear to be on the point of becoming an established component of our armamentarium in the treatment of cancer. The initial wave of anthracycline agents (DaunoXome, Doxil/Caelyx, TLC D-99) has provided proof of principle that these preparations can alter the efficacy and toxicity profiles of the parent compound. As a result of these initial successes, other agents (platins and vinca alkaloids) have entered preliminary clinical trials and new liposomal preparations of camptothecins (Koshkina et al 2000; Verschraegen et al 2000) and topoisomerase inhibitors (Colbern et al 1998b; Emerson et al 2000) are under investigation. In addition to the delivery of conventional cytotoxic drugs, there exists the possibility of encapsulating other groups of compounds such as radiation sensitizers (Harrington et al 1998), cytokines (Konno et al 1991) and immunomodulators (Malik et al 1991) within liposomes. Such possibilities are likely to ensure that the field of liposomal drug delivery will remain an active area of research for the foreseeable future.

## References


Adlakha-Hutcheon, G., Bally, M. B., Shew, C. R., Madden, T. D. (1999) Controlled destabilization of a liposomal drug delivery system enhances mitoxantrone antitumor activity. *Nat. Biotechnol.* 17: 775–779

Adler-Moore, J. (1994) AmBisome targeting to fungal infections. *Bone Marrow Transplant.* 14 (Suppl. 5): S3–S7

Allen, T. M., Chonn, A. (1987) Large unilamellar liposomes with low uptake by the reticuloendothelial system. *FEBS Lett.* 223: 42–46

Allen, T. M., Hansen, C. (1991) Pharmacokinetics of stealth versus conventional liposomes: effect of dose. *Biochim. Biophys. Acta* 1068: 133–141

Allen, T. M., Newman, M. S., Woodle, M. C., Mayhew, E., Uster, P. S. (1995) Pharmacokinetics and anti-tumor activity of vincristine encapsulated in sterically stabilized liposomes. *Int. J. Cancer* 62: 199–204

Bangham, A. D., Standish, H. M., Watkins, J. C. (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.* 13: 238–252

Batist, G., Winer, E., Navari, R., Rovira, D., Azarnia, N., TLC D-99 Study Group (1998) Decreased cardiac toxicity by TLC D-99 (liposome encapsulated doxorubicin) vs. doxorubicin in a randomised trial of metastatic breast carcinoma (MBC) *Proc. Am. Soc. Clin. Oncol.* 17: 115a (abstr. 443)

Batist, G., Rao, S. C., Ramakrishnan, G. (1999) Phase III study of liposome-encapsulated doxorubicin (TLC D-99) versus doxorubicin (DOX) in combination with cyclophosphamide (CPA) in patients with metastatic breast cancer (MBC) *Proc. Am. Soc. Clin. Oncol.* 18: 127a (abstr. 486)

Berry, G., Billingham, M., Alderman, E., Richardson, P., Torti, F., Lum, B., Patek, A., Martin, F. J. (1998) The use of cardiac biopsy to demonstrate reduced cardiotoxicity in AIDS Kaposi's sarcoma patients treated with pegylated liposomal doxorubicin. *Ann. Oncol.* 9: 711–716

Bogner, J. R., Kronawitter, U., Rolinski, B., Truebenach, K., Goebel, F.-D. (1994) Liposomal doxorubicin in the treatment of advanced AIDS-related Kaposi's sarcoma. *J. AIDS* 7: 463–468

Boman, N. L., Masin, D., Mayer, L. D., Cullis, P. R., Bally, M. B. (1994) Liposomal vincristine which exhibits increased drug retention and increased circulation longevity cures mice bearing P388 tumors. *Cancer Res.* 54: 2830–2833

Cabanes, A., Briggs, K. E., Gokhale, P. C., Treat, J. A., Rahman, A. (1998a) Comparative in-vivo studies with paclitaxel and liposome-encapsulated paclitaxel. *Int. J. Oncol.* 12: 1035–1040

Cabanes, A., Tzemach, D., Goren, D., Horowitz, A. T., Gabizon, A. (1998b) Comparative study of the antitumor activity of free doxorubicin and polyethylene glycol-coated liposomal doxorubicin in a mouse lymphoma model. *Clin. Cancer Res.* 4: 499–505

Caponigro, F., Comella, P., Budillon, A., Bryce, J., Avallone, A., De Rosa, V., Ionna, F., Comella, G. (2000) Phase I study of Caelyx (doxorubicin HCL, pegylated liposomal) in recurrent or metastatic head and neck cancer. *Ann. Oncol.* 11: 339–342

Chachoua, A., Green, M., Laubenstein, L., Wernz, J., Muggia, F. M. (1987) Phase II study of oral idarubicin in patients with AIDS-associated Kaposi's sarcoma. *Cancer Treat. Rep.* 71: 775–776

Chang, C. W., Barber, L., Ouyang, C., Masin, D., Bally, M. B., Madden, T. D. (1997) Plasma clearance, biodistribution and therapeutic properties of mitoxantrone encapsulated in conventional and sterically stabilised liposomes after intravenous administration in BDF1 mice. *Br. J. Cancer* 75: 169–177

Cheung, T. W., Remick, S. C., Azarnia, N., Proper, J. A., Barrueco, J. R., Dezube, B. J. (1999) AIDS-related Kaposi's sarcoma: a phase II study of liposomal doxorubicin. The TLC D-99 Study Group. *Clin. Cancer Res.* 5: 3432–3437

Colbern, G. T., Musterer, R., Hiller, A. J., Pegg, E., Engbers, C., Working, P. K., Henderson, I. C. (1998a) Treatment of palmar-plantar erythrodysaesthesia with pyridoxine or ergotamine: effect on efficacy of Doxil in Lewis lung carcinoma. *Proc. Am. Soc. Clin. Oncol.* 17: 238a (abstr. 913)

Colbern, G. T., Dykes, D. J., Engbers, C., Musterer, R., Hiller, A., Pegg, E., Saville, R., Weng, S., Luzzio, M., Uster, P., Amantea, M., Working, P. K. (1998b) Encapsulation of the topoisomerase I inhibitor GL147211C in pegylated (STEALTH) liposomes: pharmacokinetics and antitumor activity in HT29 colon tumor xenografts. *Clin. Cancer Res.* 4: 3077–3082

Colbern, G. T., Hiller, A. J., Musterer, R. S., Working, P. K., Henderson, I. C. (1999) Antitumor activity of Herceptin in combination with STEALTH liposomal cisplatin or nonliposomal cisplatin in a HER2 positive human breast cancer model. *J. Inorg. Biochem.* 77: 117–120

Cortes, J., O'Brien, S., Estey, E., Giles, F., Keating, M., Kantarjian, H. (1999) Phase I study of liposomal daunorubicin in patients with acute leukemia. *Invest. New Drugs* 17: 81–87

Cortes, J., Estey, E., O'Brien, S., Giles, F., Shen, Y., Koller, C., Beran, M., Thomas, D., Keating, M., Kantarjian, H. (2001) High-dose liposomal daunorubicin and high-dose cytarabine combination in patients with refractory or relapsed acute myelogenous leukemia. *Cancer* 92: 7–14

Cowens, J. W., Creaven, P. J., Greco, W. R., Brenner, D. E., Tung, Y., Ostro, M., Pilkiewicz, F., Ginsberg, R., Petrelli, N. (1993) Initial clinical (Phase I) trial of TLC D-99 (doxorubicin encapsulated in liposomes) *Cancer Res.* 53: 2796–2802

Daniele, B., De Vivo, R., Perrone, F., Lastoria, S., Tambaro, R., Izzo, F., Fiore, F., Vallone, P., Pignata, S. (2000) Phase I clinical trial of liposomal daunorubicin in hepatocellular carcinoma complicating liver cirrhosis. *Anticancer Res.* 20: 1249–1251

DeMario, M. D., Vogelzang, N. J., Janisch, L., Tonda, M., Amantea, M. A., Pendyala, L., Ratain, M. J. (1998) A phase I study of liposome-formulated cisplatin (SPI-77®) given every 3 weeks in patients with advanced cancer. *Proc. Am. Soc. Clin. Oncol.* 17: 230a (abstr. 883)

DeVore, R. F., Johnson, D. H., Strupp, J., Krozely, P., Hunt, G., Tonda, M., Wilson, C. (1999) Phase I trial of SPI-077 plus paclitaxel in patients with refractory non-small cell lung cancer (NSCLC) *Proc. Am. Soc. Clin. Oncol.* 18: 488a (abstr. 1881)

Durocher, J. R., Payne, R. C., Conrad, M. E. (1975) Role of sialic acid in erythrocyte survival. *Blood* 45: 11–20

Dvorak, J., Zoul, Z., Melichar, B., Jandik, P., Mergancova, J., Hrncirova, I., Urminska, H., Petera, J. (2001) Pegylated liposomal doxorubicin in combination with hyperthermia for treatment of skin metastases of breast carcinoma: a case report. *Onkologie* 24: 166–168

Elson, P., Chidiac, T., Budd, G. T., Pelley, R., Sandstrom, K., McLain, D., Crownover, R., Marks, K., Muschler, G., Zehr, R., Joyce, M., Bukowski, R. (1998) Phase II trial of Doxil in advanced soft tissue sarcomas (STS) *Proc. Am. Soc. Clin. Oncol.* 17: 513a (abstr. 1979)

Embree, L., Gelmon, K. A., Lohr, A., Mayer, L. D., Coldman, A. J., Cullis, P. R., Palaitis, W., Pilkiewicz, F., Hudon, N. J., Heggie, J. R. (1993) Chromatographic analysis and pharmacokinetics of liposome-encapsulated doxorubicin in non small-cell lung cancer patients. *J. Pharm. Sci.* 82: 627–634

Emerson, D. L., Bendele, R., Brown, E., Chiang, S., Desjardins, J. P., Dihel, L. C., Gill, S. C., Hamilton, M., LeRay, J. D., Moon-McDermott, L., Moynihan, K., Richardson, P. C., Tomkinson, B., Luzzio, M. J., Baccanari, D. (2000) Antitumor efficacy, pharmacokinetics, and biodistribution of NX 211: a low-clearance liposomal formulation of lurtotecan. *Clin. Cancer Res.* 6: 2903–2912

Fischl, M. A., Krown, S. E., O'Boyle, K. P., Mitsuyasu, R., Miles, S., Wernz, J. C., Volberding, P. A., Kahn, J., Groopman, J. E., Feinberg, J. (1993) Weekly doxorubicin in the treatment of patients with AIDS-related Kaposi's sarcoma. AIDS Clinical Trials Group. *J. AIDS* 6: 259–264

Forssen, E. A., Ross, M. E. (1994) DaunoXome® treatment of solid tumours; preclinical and clinical investigations. *J. Liposome Res.* 4: 481–512

Forssen, E. A., Tokes, Z. A. (1983) Improved therapeutic benefits of doxorubicin by entrapment in anionic liposomes. *Cancer Res.* 43: 546–550

Forssen, E. A., Coulter, D. M., Proffitt, R. T. (1992) Selective in-vivo localisation of daunorubicin small unilamellar vesicles in solid tumours. *Cancer Res.* 52: 3255–3261

Forssen, E. A., Male-Brune, R., Adler-Moore, J. P., Lee, M. J., Schmidt, P. G., Krasieva, T. B., Shimizu, S., Tromberg, B. J. (1996) Fluorescence imaging studies for the disposition of daunorubicin liposomes (DaunoXome) within tumour tissue. *Cancer Res.* 56: 2066–2075

Gabizon, A. A. (1992) Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. *Cancer Res.* 52: 891–896

Gabizon, A. A. (1994) Liposomal anthracyclines. *Hematol. Oncol. Clin. North Am.* 8: 431–450

Gabizon, A., Papahadjopoulos, D. (1988) Liposome formulations with prolonged circulation time in blood and enhanced uptake in tumours. *Proc. Natl Acad. Sci. USA* 85: 6949–6953

Gabizon, A., Dagan, A., Goren, D., Barenholz, Y., Fuks, Z. (1982) Liposomes as in-vivo carriers of adriamycin: reduced cardiac uptake and preserved antitumour activity in mice. *Cancer Res.* 42: 4734–4739

Gabizon, A., Goren, D., Fuks, Z., Barenholz, Y., Dagan, A., Meshorer, A. (1983) Enhancement of adriamycin delivery to liver metastatic cells with increased tumouricidal effect using liposomes as drug carriers. *Cancer Res.* 43: 4730–4735

Gabizon, A., Goren, D., Fuks, Z., Meshorer, A., Barenholz, Y. (1985) Superior therapeutic activity of liposome-associated adriamycin in a murine metastatic tumour model. *Br. J. Cancer* 51: 681–689

Gabizon, A., Meshorer, A., Barenholz, Y. (1986) Comparative long-term study of the toxicities of free and liposome-associated doxorubicin in mice after intravenous administration. *J. Natl Cancer Inst.* 77: 459–469

Gabizon, A., Shiota, R., Papahadjopoulos, D. (1989) Pharmacokinetics and tissue distribution of doxorubicin encapsulated in stable liposomes with long circulation times. *J. Natl Cancer Inst.* 81: 1484–1488

Gabizon, A., Price, D. C., Huberty, J., Bresalier, R. S., Papahadjopoulos, D. (1990) Effect of liposome composition and other factors on the targeting of liposomes to experimental tumours: biodistribution and imaging studies. *Cancer Res.* 50: 6371–6378

Gabizon, A., Chisin, R., Amselem, S., Druckmann, S., Cohen, R., Goren, D., Fromer, I., Peretz, T., Sulkes, A., Barenholz, Y. (1991) Pharmacokinetic and imaging studies in patients receiving a formulation of liposome-associated adriamycin. *Br. J. Cancer* 64: 1125–1132

Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Safra, T., Cohen, R., Martin, F., Huang, A., Barenholz, Y. (1994) Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polethylene-glycol coated liposomes. *Cancer Res.* 54: 987–992

Gelmon, K. A., Tolcher, A., Diab, A. R., Bally, M. B., Embree, L., Hudon, N., Dedhar, C., Ayers, D., Eisen, A., Melosky, B., Burge, C., Logan, P., Mayer, L. D. (1999) Phase I study of liposomal vincristine. *J. Clin. Oncol.* 17: 697–705

Gill, P. S., Espina, B. M., Muggia, F., Cabriales, S., Tulpule, A., Esplin, J. A., Liebman, H. A., Forssen, E., Ross, M. E., Levine, A. M. (1995) Phase I/II clinical and pharmacokinetic evaluation of liposomal daunorubicin. *J. Clin. Oncol.* 13: 996–1003

Gill, P. S., Wernz, J., Scadden, D. T., Cohen, P., Mukwaya, G. M., von Roenn, J. H., Jacobs, M., Kempin, S., Silverberg, I., Gonzales, G., Rarick, M. U., Myers, A. M., Shepherd, F., Sawka, C., Pike, M. C., Ross, M. E. (1996) Randomised phase III trial of liposomal daunorubicin versus doxorubicin, bleomycin and vincristine in AIDS-related Kaposi's sarcoma. *J. Clin. Oncol.* **14**: 2353–2364

Girard, P. M., Bouchaud, O., Goetschel, A., Mukwaya, G., Eestermans, G., Ross, M., Rozenbaum, W., Saimot, A. G. (1996) Phase II study of liposomal encapsulated daunorubicin in the treatment of AIDS-associated mucocutaneous Kaposi's sarcoma. *AIDS* **10**: 753–757

Goebel, F.-D., Goldstein, D., Goos, M., Jablonowski, H., Stewart, J. S. (1996) Efficacy and safety of Stealth liposomal doxorubicin in AIDS-related Kaposi's sarcoma. *Br. J. Cancer* **73**: 989–994

Gokhale, P. C., Radhakrishnan, B., Husain, S. R., Abernethy, D. R., Sacher, R., Dritschilo, A., Rahman, A. (1996) An improved method of encapsulation of doxorubicin in liposomes: pharmacological, toxicological and therapeutic evaluation. *Br. J. Cancer* **74**: 43–48

Gondal, J. A., Preuss, H. G., Swartz, R., Rahman, A. (1993) Comparative pharmacological, toxicological and antitumoural evaluation of free and liposome-encapsulated cisplatin in rodents. *Eur. J. Cancer* **29A**: 1536–1542

Gordon, K. B., Tajuddin, A., Guitart, J., Kuzel, T. M., Eramo, L. R., Von Roenn, J. (1995) Hand-foot syndrome associated with liposome-encapsulated doxorubicin therapy. *Cancer* **75**: 2169–2173

Gordon, A. N., Granai, C. O., Rose, P. G., Hainsworth, J., Lopez, A., Weissman, C., Rosales, R., Sharpington, T. (2000) Phase II study of liposomal doxorubicin in platinum- and paclitaxel-refractory epithelial ovarian cancer. *J. Clin. Oncol.* **18**: 3093–3100

Gregoriadis, G. (1976a) The carrier potential of liposomes in biology and medicine (first of two parts) *N. Engl. J. Med.* **295**: 704–710

Gregoriadis, G. (1976b) The carrier potential of liposomes in biology and medicine (second of two parts) *N. Engl. J. Med.* **295**: 765–770

Gregoriades, G., Swain, C. P., Wills, E. J., Tavill, A. S. (1974) Drug-carrier potential of liposomes in cancer chemotherapy. *Lancet* **1**: 1313–1316

Halford, S., Yip, D., Karapetis, C. S., Strickland, A. H., Steger, A., Khawaja, H. T., Harper, P. G. (2001) A phase II study evaluating the tolerability and efficacy of CAELYX (liposomal doxorubicin, Doxil) in the treatment of unresectable pancreatic carcinoma. *Ann. Oncol.* **12**; 1399–1402

Halm, U., Etzrodt, G., Schiefke, I., Schmidt, F., Witzigmann, H., Mossner, J., Berr, F. (2000) A phase II study of pegylated liposomal doxorubicin for treatment of advanced hepatocellular carcinoma. *Ann. Oncol.* **11**: 113–114

Harasym, T. O., Cullis, P. R., Bally, M. B. (1997) Intratumour distribution of doxorubicin following i.v. administration of drug encapsulated in egg phosphatidylcholine/cholesterol liposomes. *Cancer Chemother. Pharmacol.* **40**: 309–317

Harrington, K. J., Rowlinson-Busza, G., Uster, P. S., Whittaker, J., Stewart, J. S. W. (1998) Stealth liposome encapsulated doxorubicin (SLED) and iododeoxyuridine (SLIUDR) as radiation sensitisers in head and neck squamous cell cancer xenograft tumours (HNSCCXT) *Proc. Am. Soc. Clin. Oncol.* **17**: 230a (abstr. 881)

Harrington, K. J., Rowlinson-Busza, G., Syrigos, K. N., Uster, P. S., Abra, R. M., Stewart, J. S. (2000a) Biodistribution and pharmacokinetics of $^{111}$In-DTPA-labelled pegylated liposomes in a human tumour xenograft model: implications for novel targeting strategies. *Br. J. Cancer* **83**: 232–238

Harrington, K. J., Rowlinson-Busza, G., Syrigos, K. N., Abra, R. M., Uster, P. S., Peters, A. M., Stewart, J. S. (2000b) Influence of tumour size on uptake of $^{111}$In-DTPA-labelled pegylated liposomes in a human tumour xenograft model. *Br. J. Cancer* **83**: 684–688

Harrington, K. J., Rowlinson-Busza, G., Uster, P. S., Stewart, J. S. (2000c) Pegylated liposome-encapsulated doxorubicin and cisplatin in the treatment of head and neck xenograft tumours. *Cancer Chemother. Pharmacol.* **46**: 10–18

Harrington, K. J., Mohammadtaghi, S., Uster, P. S., Glass, D. M., Peters, A. M., Vile, R. G., Stewart, J. S. W. (2001a) Effective targeting of solid tumours in patients with locally advanced cancers by radiolabelled pegylated liposomes. *Clin. Cancer Res.* **7**: 243–254

Harrington, K. J., Lewanski, C. R., Northcote, A. D., Whittaker, J., Vile, R. G., Peters, A. M., Stewart, J. S. W. (2001b) Phase II study of pegylated liposomal doxorubicin (Caelyx™) in patients with inoperable, locally advanced squamous cell cancer of the head and neck. *Eur. J. Cancer* **37**: 2015–2022

Harrington, K. J., Lewanski, C. R., Northcote, A. D., Whittaker, J., Wellbank, H., Vile, R. G., Peters, A. M., Stewart, J. S. W. (2001c) Phase I/II study of pegylated liposomal cisplatin (SPI-077™) in patients with inoperable head and neck cancer. *Ann. Oncol.* **12**: 493–496

Harris, L., Winer, E., Batist, G., Rovira, D., Navari, R., Lee, L., TLC D-99 Study Group. (1998) Phase III study of TLC D-99 (liposome encapsulated doxorubicin) vs. free doxorubicin (Dox) in patients with metastatic breast carcinoma (MBC) *Proc. Am. Soc. Clin. Oncol.* **17**: 124a (abstr. 474)

Harrison, M., Tomlinson, D., Stewart, S. (1995) Liposomal entrapped doxorubicin: an active agent in AIDS-related Kaposi's sarcoma. *J. Clin. Oncol.* **13**: 914–920

Hengge, U. R., Brockmeyer, N. H., Baumann, M., Reimann, G., Goos, M. (1993) Liposomal doxorubicin in AIDS-related Kaposi's sarcoma. *Lancet* **343**: 497

Hong, R. L., Huang, C. J., Tseng, Y. L., Pang, V. F., Chen, S. T., Liu, J. J., Chang, F. H. (1999) Direct comparison of liposomal doxorubicin with or without polyethylene glycol coating in C-26 tumor-bearing mice: is surface coating with polyethylene glycol beneficial? *Clin. Cancer Res.* **5**: 3645–3652

Huang, S. K., Mayhew, E., Gilani, S., Lasic, D. D., Martin, F. J., Papahadjopoulos, D. (1992a) Pharmacokinetics and therapeutics of sterically stabilised liposomes in mice bearing C-26 colon carcinoma. *Cancer Res.* **52**: 6774–6781

Huang, S. K., Lee, K.-D., Hong, K., Friend, D. S., Papahadjopoulos, D. (1992b) Microscopic localisation of sterically stabilised liposomes in colon carcinoma-bearing mice. *Cancer Res.* **52**: 5135–5143

Huang, S. K., Martin, F. J., Jay, G., Vogel, J., Papahadjopoulos, D., Friend, D. S. (1993) Extravasation and transcytosis of liposomes in Kaposi's sarcoma-like dermal lesions of transgenic mice bearing the HIV*tat* gene. *Am. J. Pathol.* **143**: 1–14

Ishida, O., Maruyama, K., Sasaki, K., Iwatsuru, M. (1999) Size-dependent extravasation and interstitial localization of polyethyleneglycolliposomes in solid tumor-bearing mice. *Int. J. Pharm.* **190**: 49–56

Israel, V. K., Jeffers, S., Bernal, G., Rueda, G., Roman, L., Roman, C. P., Rosales, R., Amantea, M., Muggia, F. M. (1998) Phase I study of Doxil (liposomal doxorubicin) in combination with paclitaxel. *Proc. Am. Soc. Clin. Oncol.* **17**: 245a (abstr. 938)

Israel, V. P., Garcia, A. A., Roman, L., Muderspach, L., Burnett, A., Jeffers, S., Muggia, F. M. (2000) Phase II study of liposomal doxorubicin in advanced gynecologic cancers. *Gynecol. Oncol.* **78**: 143–147

James, N. D., Coker, R. J., Tomlinson, D., Harris, J. R., Gompels, M., Pinching, A. J., Stewart, J. S. (1994) Liposomal doxorubicin (Doxil): an effective new treatment for Kaposi's sarcoma in AIDS. *Clin. Oncol. (R. Coll. Radiol.)* **6**: 294–296

Johnston, S. R., Gore, M. E. (2001) Caelyx: phase II studies in ovarian cancer. *Eur. J. Cancer* **37**; S8–S14

Judson, I., Radford, J. A., Harris, M., Blay, J. Y., van Hoesel, Q., le Cesne, A., van Oosterom, A. T., Clemons, M. J., Kamby, C., Hermans, C., Whittaker, J., Donato di Paola, E., Verweij, J., Nielsen, S. (2001) Randomised phase II trial of pegylated liposomal

doxorubicin (DOXIL/CAELYX) versus doxorubicin in the treatment of advanced or metastatic soft tissue sarcoma: a study by the EORTC Soft Tissue and Bone Sarcoma Group. *Eur. J. Cancer* **37**: 870–877

Kaplan, L., Volberding, P. A. (1985) Failure (and danger) of mitozantrone in AIDS-related Kaposi's sarcoma. *Lancet* **2**: 396

Khalifa, A., Dodds, D., Rampling, R., Paterson, J., Murray, T. (1997) Liposomal distribution in malignant glioma: possibilities for therapy. *Nucl. Med. Commun.* **18**: 17–23

Khokhar, A. R., Wright, K., Siddik, Z. H., Perez-Soler, R. (1988) Organ distribution and tumour uptake of liposome entrapped *cis*-bis-neodecanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II) administered intravenously and into the proper hepatic artery. *Cancer Chemother. Pharmacol.* **22**: 223–227

Khokhar, A. R., al-Baker, S., Krakoff, I. H., Perez-Soler, R. (1989) Toxicity and antitumour activity of *cis*-bis-carboxylato(*trans-R,R*-1,2-diaminocyclohexane) platinum (II) complexes entrapped in liposomes. *Cancer Chemother. Pharmacol.* **23**: 219–224

Klein, P., Lyass, O., Sorich, J. (1998) Doxil plus cisplatin: combined results of two Phase I studies. *Proc. Am. Soc. Clin. Oncol.* **17**: 246a (abstr. 942)

Klibanov, A. L., Maruyama, K., Torchilin, V. P., Huang, L. (1990) Amphipathic polyethyleneglycols effectively prolong the circulation times of liposomes. *FEBS Lett.* **268**: 235–237

Konno, H., Yamashita, A., Tadakuma, T., Sakaguchi, S. (1991) Inhibition of growth of rat hepatoma by local injection of liposomes containing recombinant interleukin-2. Antitumour effect of IL-2 liposome. *Biotherapy* **3**: 211–218

Koshkina, N. V., Kleinerman, E. S., Waidrep, C., Jia, S. F., Worth, L. L., Gilbert, B. E., Knight, V. (2000) 9-Nitrocamptothecin liposome aerosol treatment of melanoma and osteosarcoma lung metastases in mice. *Clin. Cancer Res.* **6**: 2876–2880

Koukourakis, M. I., Koukouraki, S., Giatromanolaki, A., Archimandritis, S. C., Skarlatos, J., Beroukas, K., Bizakis, J. G., Retalis, G., Karkavitsas, N., Helidonis, E. S. (1999) Liposomal doxorubicin and conventionally fractionated radiotherapy in the treatment of locally advanced non-small-cell lung cancer and head and neck cancer. *J. Clin. Oncol.* **17**: 3512–3521

Kouloulias, V. E., Dardoufas, C. E., Kouvaris, J. R., Gennatas, C. S., Polyzos, A. K., Gogas, H. J., Sandilos, P. H., Uzunoglu, N. K., Malas, E. G., Vlahos, L. J. (2002) Liposomal doxorubicin in conjunction with reirradiation and local hyperthermia treatment in recurrent breast cancer: a phase I/II trial. *Clin. Cancer Res.* **8**: 374–382

Kubo, A., Nakamura, K., Sammiya, T., Katayama, M., Hashimoto, T., Hashimoto, S., Kobayashi, H., Teramoto, T. (1993) Indium-111-labelled liposomes: dosimetry and tumour detection in patients with cancer. *Eur. J. Nucl. Med.* **20**: 107–113

Langley, R. E., Carmichael, J., Woll, P. J., Mason, E., Welbank, H. (1998) Phase II trial to evaluate the safety and tolerability of Caelyx® in combination with paclitaxel in the treatment of metastatic cancer. *Proc. Am. Soc. Clin. Oncol.* **17**: 246a (abstr. 943)

Lasic, D. D. (1996) Doxorubicin in sterically stabilised liposomes. *Nature* **380**: 561–562

Lasic, D. D., Papahadjopoulos, D. (1995) Liposomes revisited. *Science* **267**: 1275–1276

Laufman, L. R., Spiridonidis, C. H., Jones, J. J., Rhodes, V. A., Wallace, K. (1998) Phase I trial of Doxil® plus vinorelbine (VNB) in patients (PTS) with advanced malignancies. *Proc. Am. Soc. Clin. Oncol.* **17**: 246a (abstr. 944)

Leyland-Jones, B. (1993) Targeted drug delivery. *Semin. Oncol.* **20**: 12–17

Lippens, R. J. (1999) Liposomal daunorubicin (DaunoXome) in children with recurrent or progressive brain tumors. *Pediatr. Hematol. Oncol.* **16**: 131–139

Lopez, A. M., Wallace, L., Dorr, R. T., Koff, M., Hersh, E. M., Alberts, D. S. (1999) Topical DMSO treatment for pegylated liposomal doxorubicin-induced palmar-plantar erythrodysesthesia. *Cancer Chemother. Pharmacol.* **44**: 303–306

Lotem, M., Hubert, A., Lyass, O., Goldenhersh, M. A., Ingber, A., Peretz, T., Gabizon, A. (2000) Skin toxic effects of polyethylene glycol-coated liposomal doxorubicin. *Arch. Dermatol.* **136**: 1475–1480

Lyass, O., Uziely, B., Ben-Yosef, R., Tzemach, D., Heshing, N. I., Lotem, M., Brufman, G., Gabizon, A. (2000) Correlation of toxicity with pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in metastatic breast carcinoma. *Cancer* **89**: 1037–1047

Madhavan, S., Northfelt, D. W. (1995) Lack of vesicant injury following extravasation of liposomal doxorubicin. *J. Natl Cancer Inst.* **87**: 1556–1557

Malik, S. T., Martin, D., Hart, I., Balkwill, F. (1991) Therapy of human ovarian cancer xenografts with intraperitoneal liposome encapsulated muramyl-tripeptide phosphoethanolamine (MTP-PE) and recombinant GM-CSF. *Br. J. Cancer* **63**: 399–403

Malik, U. R., Sparano, J. A., Wolffe, A. (1998) Phase I trial of liposomal doxorubicin (Doxil) and docetaxel (Taxotere) in patients (PTS) with advanced breast cancer (ABC) *Proc. Am. Soc. Clin. Oncol.* **17**: 175a (abstr. 672)

Markman, M., Kennedy, A., Webster, K., Peterson, G., Kulp, B., Belinson, J. (2000) Phase 2 trial of liposomal doxorubicin (40 mg/m$^2$) in platinum/paclitaxel-refractory ovarian and fallopian tube cancers and primary carcinoma of the peritoneum. *Gynecol. Oncol.* **78**: 369–372

Martin, F. J. (1997) Pegylated liposomal doxorubicin: scientific rationale and preclinical pharmacology. *Oncology* **11** (Suppl. 11): 11–20

Mavroudis, D., Kouroussis, C., Kakolyris, S., Agelaki, S., Kalbakis, K., Androulakis, N., Souglakos, J., Samonis, G., Georgoulias, V. (2002) Phase I study of paclitaxel (taxol) and pegylated liposomal doxorubicin (caelyx) administered every 2 weeks in patients with advanced solid tumors. *Oncology* **62**; 216–222

Mayer, L. D., Bally, M. B., Loughrey, H., Masin, D., Cullis, P. R. (1990) Liposomal vincristine preparations which exhibit decreased drug toxicity and increased activity against murine L1210 and P388 tumors. *Cancer Res.* **50**: 575–579

Mayhew, E. G., Goldrosen, M. H., Vaage, J., Rustum, Y. M. (1987) Effects of liposome-entrapped doxorubicin on liver metastases of mouse colon carcinomas 26 and 38. *J. Natl Cancer Inst.* **78**: 707–713

Mayhew, E. G., Lasic, D., Babbar, S., Martin, F. J. (1992) Pharmacokinetics and antitumour activity of epirubicin encapsulated in long-circulating liposomes incorporating a polyethylene glycol-derivatized phospholipid. *Int. J. Cancer* **51**: 302–309

Money-Kyrle, J. F., Bates, F., Ready, J., Gazzard, B. G., Phillips, R. H., Boag, F. C. (1993) Liposomal daunorubicin in advanced Kaposi's sarcoma: a phase II study. *Clin. Oncol. (R. Coll. Radiol.)* **5**: 367–371

Mori, A., Wu, S. P., Han, I., Khokhar, A. R., Perez-Soler, R., Huang, L. (1996) In-vivo antitumour activity of *cis*-bis-neodecanoato-*trans-R,R*-1,2-diaminocyclohexane platinum(II) formulated in long-circulating liposomes. *Cancer Chemother. Pharmacol.* **37**: 435–444

Muggia, F., Hamilton, A. (2001) Phase III data on Caelyx in ovarian cancer. *Eur. J. Cancer* **37** (Suppl. 9): S15–S18

Muggia, F. M., Hainsworth, J. D., Jeffers, S., Miller, P., Groshen, S., Tan, M., Roman, L., Uziely, B., Muderspach, L., Garcia, A., Burnett, A., Greco, F. A., Morrow, C. P., Paradiso, L. J., Liang, L. J. (1997) Phase II study of liposomal doxorubicin in refractory ovarian cancer: antitumour activity and toxicity modification by liposomal encapsulation. *J. Clin. Oncol.* **15**: 987–993

Newman, M. S., Colbern, G. T., Working, P. K., Engbers, C., Amantea, M. A. (1999) Comparative pharmacokinetics, tissue distribution and therapeutic effectiveness of cisplatin encapsulated in

long-circulating, pegylated liposomes (SPI-077) in tumour-bearing mice. *Cancer Chemother. Pharmacol.* **43**: 1–7

Ng, T. T., Denning, D. W. (1995) Liposomal amphotericin B (AmBisome) therapy in invasive fungal infections. Evaluation of United Kingdom compassionate use data. *Arch. Intern. Med.* **155**: 1093–1098

Northfelt, D. W., Martin, F. J., Working, P., Volberding, P. A., Russell, J., Newman, M., Amantea, M. A., Kaplan, L. D. (1996) Doxorubicin encapsulated in liposomes containing surface-bound polyethylene glycol: pharmacokinetics, tumour localisation, and safety in patients with AIDS-related Kaposi's sarcoma. *J. Clin. Pharmacol.* **36**: 55–63

Northfelt, D. W., Dezube, B. J., Thommes, J. A., Miller, B. J., Fischl, M. A., Friedman-Kien, A., Kaplan, L. D., Du Mond, C., Mamelok, R. D., Henry, D. H. (1998) Pegylated-liposomal doxorubicin versus doxorubicin, bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma: results of a randomised phase III clinical trial. *J. Clin. Oncol.* **16**: 2445–2451

O'Brien, M., Hickish, T., Iveson, T., Gilligan, D., Harper, P., Light, L., Whittaker, J., Smith, I. E. (1998) Phase II study of Caelyx® (Doxil®; doxorubicin HCl pegylated liposomal) in the treatment of small cell lung cancer (SCLC). *Proc. Am. Soc. Clin. Oncol.* **17**: 497a (abstr. 1913)

O'Byrne, K. J., Thomas, A. L., Sharma, R. A., DeCatris, M., Shields, F., Beare, S., Steward, W. P. (2002) A phase I dose-escalating study of DaunoXome, liposomal daunorubicin, in metastatic breast cancer. *Br. J. Cancer* **87**; 15–20

Ogihara-Umeda, I., Sasaki, T., Kojima, S., Nishigori, H. (1996) Optimal radiolabelled liposomes for tumour imaging. *J. Nucl. Med.* **37**: 326–332

Papahadjopoulos, D., Allen, T. M., Gabizon, A., Mayhew, E., Matthay, K., Huang, S. K., Lee, K. D., Woodle, M. C., Lasic, D. D., Redemann, C. (1991) Sterically stabilised liposomes: improvements in pharmacokinetics and anti-tumour therapeutic efficacy. *Proc. Natl Acad. Sci. USA* **88**: 11460–11464

Pennington, K., Gordon, M., Picus, J. (1998) A phase II trial of liposomal doxorubicin (Doxil®) in the treatment of advanced renal cell cancer: a Hoosier Oncology Group (HOG) study. *Proc. Am. Soc. Clin. Oncol.* **17**: 339a (abstr. 1308)

Perez-Soler, R. (1989) Liposomes as carriers of antitumour agents: toward a clinical reality. *Cancer Treat. Rev.* **16**: 67–82

Perez-Soler, R., Khokhar, A. R., Lopez-Berenstein, G. (1987) Treatment and prophylaxis of experimental liver metastases of M5076 reticulosarcoma with *cis*-bis-neodecanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II) entrapped in multilamellar vesicles. *Cancer Res.* **47**: 6462–6466

Perez-Soler, R., Young, L. Y., Drewinko, B., Lautersztain, J., Khokhar, A. R. (1988) Increased cytotoxicity and reversal of resistance to cisplatin with entrapment of *cis*-bis-neodecanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II) in multilamellar vesicles. *Cancer Res.* **48**: 4509–4512

Perez-Soler, R., Lopez-Berestein, G., Lautersztain, J., al-Baker, S., Francis, K., Macias-Kiger, D., Raber, M. N., Khokhar, A. R. (1990) Phase I clinical and pharmacology study of liposome-entrapped *cis*-bis-neodecanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II) *Cancer Res.* **50**: 4254–4259

Perez-Soler, R., Shin, D. M., Siddik, Z. H., Murphy, W. K., Huber, M., Lee, S. J., Khokhar, A. R., Hong, W. K. (1997) Phase I clinical and pharmacological study of liposome-entrapped NDDP administered intrapleurally in patients with malignant pleural effusions. *Clin. Cancer Res.* **3**: 373–379

Perez-Soler, R., Walsh, G. L., Swisher, S. G. (1999) Phase II study of liposome-entrapped cisplatin-analog (L-NDDP) administered intrapleurally in patients with malignant pleural mesothelioma. *Proc. Am. Soc. Clin. Oncol.* **18**: 421a (abstr. 1626)

Prentice, H. G., Hann, I. M., Herbrecht, R., Aoun, M., Kvaloy, S., Catovsky, D., Pinkerton, C. R., Schey, S. A., Jacobs, F., Oakhill, A., Stevens, R. F., Darbyshire, P. J., Gibson, B. E. (1997) A randomised comparison of liposomal versus conventional amphotericin B for the treatment of pyrexia of unknown origin in neutropenic patients. *Br. J. Haematol.* **98**: 711–718

Presant, C. A., Proffitt, R. T., Turner, A. F., Williams, L. E., Winsor, D., Werner, J. L., Kennedy, P., Wiseman, C., Gala, K., McKenna. R. J. (1988) Successful imaging of human cancer with Indium-111-labelled phospholipid vesicles. *Cancer* **62**: 905–911

Presant, C. A., Blayney, D., Proffitt, R. T., Turner, A. F., Williams, L. E., Nadel, H. I., Kennedy, P., Wiseman, C., Gala, K., Crossley, R. J. (1990) Preliminary report: imaging of Kaposi sarcoma and lymphoma in AIDS with indium-111-labelled liposomes. *Lancet* **335**: 1307–1309

Presant, C. A., Scolaro, M., Kennedy, P., Blayney, D. W., Flanagan, B., Lisak, J., Presant, J. (1993) Liposomal daunorubicin treatment of HIV-associated Kaposi's sarcoma. *Lancet* **341**: 1242–1243

Proffitt, R. T., Williams, L. E., Presant, C. A., Tin, G. W., Uliana, J. A., Gamble, R. C., Baldeschwieler, J. D. (1983) Tumour-imaging potential of liposomes loaded with In-111-NTA: biodistribution in mice. *J. Nucl. Med.* **24**: 45–51

Rahman, A., Kessler, A., More, N., Sikic, B., Rowden, G., Woolley, P., Schein, P. S. (1980) Liposomal protection of adriamycin-induced cardiotoxicity in mice. *Cancer Res.* **40**: 1532–1537

Ramirez, M. R., Marcom, P. K., Sutton, L. M., Harris, L. N., Petros, W. P., Berry, D. A., Warmuth, M. A., Winer, E. P. (1998) A phase I/II study of Doxil and vinorelbine in metastatic breast cancer patients. *Proc. Am. Soc. Clin. Oncol.* **17**: 169a (abstr. 650)

Ranson, M. R., Carmichael, J., O'Byrne, K., Stewart, S., Smith, D., Howell, A. (1997) Treatment of advanced breast cancer with sterically stabilized liposomal doxorubicin: results of a multicentre phase II trial. *J. Clin. Oncol.* **15**, 3185–3191

Richardson, V. J., Ryman, B. E., Jewkes, R. F., Jeyasingh, K., Tattersall, M. N., Newlands, E. S., Kaye, S. B. (1979) Tissue distribution and tumour localisation of 99m-technetium-labelled liposomes in cancer patients. *Br. J. Cancer* **40**: 35–43

Rudolph, R., Stein, R. S., Pattillo, R. A. (1976) Skin ulcers due to adriamycin. *Cancer* **38**: 1087–1094

Russo, R., Nigro, L. C., Minniti, S. (1996) Visceral leishmaniasis in HIV infected patients: treatment with high dose liposomal amphotericin B (AmBisome) *J. Infect.* **32**: 133–137

Sarris, A. H., Hagemeister, F., Romaguera, J., Rodriguez, M. A., McLaughlin, P., Tsimberidou, A. M., Medeiros, L. J., Samuels, B., Pate, O., Oholendt, M., Kantarjian, H., Burge, C., Cabanillas, F. (2000) Liposomal vincristine in relapsed non-Hodgkin's lymphomas: early results of an ongoing phase II trial. *Ann. Oncol.* **11**: 69–72

Schwonzen, M., Kurbacher, C. M., Mallmann, P. (2000) Liposomal doxorubicin and weekly paclitaxel in the treatment of metastatic breast cancer. *Anticancer Drugs* **11**: 681–685

Senior, J., Gregoriadis, G. (1982a) Is half-life of circulating liposomes determined by changes in their permeability? *FEBS Lett.* **145**: 109–114

Senior, J., Gregoriadis, G. (1982b) Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of the phospholipid and cholesterol components. *Life Sci.* **30**: 2123–2136

Sessa, G., Weissmann, G. (1968) Phospholipid spherules (liposomes) as a model for biological membranes. *J. Lipid Res.* **9**: 310–318

Shapiro, C. L., Ervin, T., Welles, L., Azarnia, N., Keating, J., Hayes, D. F. (1999) Phase II trial of high-dose liposome-encapsulated doxorubicin with granulocyte colony-stimulating factor in metastatic breast cancer. TLC D-99 Study Group. *J. Clin. Oncol.* **17**: 1435–1441

Shepherd, F. A., Burkes, R., Paul, K. E., Goss, P. E. (1991) A phase II study of 4'-epirubicin in the treatment of poor-risk Kaposi's sarcoma and AIDS. *AIDS* 5: 305

Shields, A. F., Lange, L. M., Zalupski, M. M. (2001) Phase II study of liposomal doxorubicin in patients with advanced colorectal cancer. *Am. J. Clin. Oncol.* 24: 96–98

Siegal, T., Horowitz, A., Gabizon, A. (1995) Doxorubicin encapsulated in sterically stabilised liposomes for the treatment of a brain tumour model: biodistribution and therapeutic efficacy. *J. Neurosurg.* 83: 1029–1037

Simpson, J. K., Miller, R. F., Spittle, M. F. (1993) Liposomal doxorubicin for the treatment of AIDS-related Kaposi's sarcoma. *Clin. Oncol. (R. Coll. Radiol.)* 5: 372–374

Skubitz, K. M. (1998) Early results of pegylated liposomal doxorubicin (Doxil®) in refractory sarcoma. *Proc. Am. Soc. Clin. Oncol.* 17: 524a (abstr. 2013)

Speyer, J., Wasserheit, C. (1998) Strategies for reduction of anthracycline cardiac toxicity. *Semin. Oncol.* 25: 525–537

Steele, J. P., O'Doherty, C. A., Shamash, J., Evans, M. T., Gower, N. H., Tischkowitz, M. D., Rudd, R. M. (2001) Phase II trial of liposomal daunorubicin in malignant pleural mesothelioma. *Ann. Oncol.* 12: 497–499

Steerenberg, P. A., Storm, G., de Groot, G., Claessen, A., Bergers, J. J., Franken, M. A., van Hoesel, Q. G., Wubs, K. L., de Jong, W. H. (1988) Liposomes as drug carrier system for cis-diammine-dichloroplatinum (II) II. Antitumour activity in-vivo, induction of drug resistance, nephrotoxicity and Pt distribution. *Cancer Chemother. Pharmacol.* 21: 299–307

Stewart, S., Jablonowski, H., Goebel, F.-D., Arasteh, K., Spittle, M., Rios, A., Aboulafia, D., Galleshaw, J., Dezube, B. J. (1998) Randomised comparative trial of pegylated liposomal doxorubicin versus bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma. International Pegylated Liposomal Doxorubicin Study Group. *J. Clin. Oncol.* 16: 683–691

Symon, Z., Peyser, A., Tzemach, D., Lyass, O., Sucher, E., Shezen, E., Gabizon, A. (1999) Selective delivery of doxorubicin to patients with breast carcinoma metastases by stealth liposomes. *Cancer* 86: 72–78

Thomas, A. L., O'Byrne, K., Furber, L., Jeffery, K., Steward, W. P. (2001) A phase II study of caelyx, liposomal doxorubicin: lack of activity in patients with advanced gastric cancer. *Cancer Chemother. Pharmacol.* 48: 266–268

Toma, S., Tucci, A., Villani, G., Carteni, G., Spadini, N., Palumbo, R. (2000) Liposomal doxorubicin (Caelyx) in advanced pretreated soft tissue sarcomas: a phase II study of the Italian Sarcoma Group (ISG) *Anticancer Res.* 20: 485–491

Tsiara, S. N., Kapsali, E., Christou, L., Panteli, A., Pritsivelis, N., Bourantas, K. L. (2000) Administration of a modified chemotherapeutic regimen containing vincristine, liposomal doxorubicin and dexamethasone to multiple myeloma patients: preliminary data. *Eur. J. Haematol.* 65: 118–122

Turner, A. F., Presant, C. A., Proffitt, R. T., Williams, L. E., Winsor, D. W., Werner, J. L. (1988) In-111-labelled liposomes: dosimetry and tumour depiction. *Radiology* 166: 761–765

Uthayakumar, S., Bower, M., Money-Kyrle, J., Muyshondt, C., Youle, M., Hannon, F., Phillips, R., Gazzard, B. G., Boag, F. (1996) Randomized cross-over comparison of liposomal daunorubicin versus observation for early Kaposi's sarcoma. *AIDS* 10: 515–519

Vaage, J., Mayhew, E., Lasic, D., Martin, F. (1992) Therapy of primary and metastatic mouse mammary carcinomas with doxorubicin encapsulated in long circulating liposomes. *Int. J. Cancer* 51: 942–948

Vaage, J., Donovan, D., Mayhew, E., Uster, P., Woodle, M. (1993a) Therapy of mouse mammary carcinomas with vincristine and doxorubicin encapsulated in sterically stabilised liposomes. *Int. J. Cancer* 54: 959–964

Vaage, J., Donovan, D., Mayhew, E., Abra, R., Huang, A. (1993b) Therapy of human ovarian carcinoma xenografts using doxorubicin encapsulated in sterically stabilised liposomes. *Cancer* 72: 3671–3675

Vaage, J., Barbera-Guillem, E., Abra, R., Huang, A., Working, P. (1994) Tissue distribution and therapeutic effect of intravenous free or encapsulated liposomal doxorubicin on human prostate carcinoma xenografts. *Cancer* 73: 1478–1484

Vaage, J., Donovan, D., Loftus, T., Working, P. (1995) Prevention of metastasis from mouse mammary carcinomas with liposomes carrying doxorubicin. *Br. J. Cancer* 72: 1074–1075

Vaage, J., Donovan, D., Uster, P., Working, P. (1997) Tumour uptake of doxorubicin in polyethylene glycol-coated liposomes and therapeutic effect against a xenografted human pancreatic carcinoma. *Br. J. Cancer* 75: 482–486

Vaage, J., Donovan, D., Wipff, E., Abra, R., Colbern, G., Uster, P., Working, P. (1999) Therapy of a xenografted human colonic carcinoma using cisplatin or doxorubicin encapsulated in long-circulating pegylated stealth liposomes. *Int. J. Cancer* 80: 134–137

Vadiei, K., Siddik, Z. H., Khokhar, A. R., al-Baker, S., Sampedro, F., Perez-Soler, R. (1992) Pharmacokinetics of liposome-entrapped *cis*-bis-neodecanoato *trans-R,R*-1,2 diaminocyclohexane platinum (II) and cisplatin given i.v. and i.p. in the rat. *Cancer Chemother. Pharmacol.* 30: 365–369

Vail, D. M., Chun, R., Thamm, D. H., Garrett, L. D., Cooley, A. J., Obradovich, J. E. (1998) Efficacy of pyridoxine to ameliorate the cutaneous toxicity associated with doxorubicin containing pegylated (Stealth) liposomes: a randomized, double-blind clinical trial using a canine model. *Clin. Cancer Res.* 4: 1567–1571

Valero, V., Buzdar, A. U., Theriault, R. L., Azarnia, N., Fonseca, G. A., Willey, J., Ewer, M., Walters, R. S., Mackay, B., Podoloff, D., Booser, D., Lee, L. W., Hortobagyi, G. N. (1999) Phase II trial of liposome-encapsulated doxorubicin, cyclophosphamide, and fluorouracil as first-line therapy in patients with metastatic breast cancer. *J. Clin. Oncol.* 17: 1425–1434

van Hoesel, Q. G., Steerenberg, P. A., Crommelin, D. J., van Dijk, A., van Oort, W., Klein, S., Douze, J. M., de Wildt, D. J., Hillen, F. C. (1984) Reduced cardiotoxicity and nephrotoxicity with preservation of anti-tumour activity of doxorubicin entrapped in stable liposomes in the LIEU/M Wsl rat. *Cancer Res.* 44: 3698–3703

Verschraegen, C. F., Gilbert, B. E., Huaringa, A. J., Newman, R., Harris, N., Leyva, F. J., Keus, L., Campbell, K., Nelson-Taylor, T., Knight, V. (2000) Feasibility, phase I, and pharmacological study of aerosolized liposomal 9-nitro-20(S)-camptothecin in patients with advanced malignancies in the lungs. *Ann. N. Y. Acad. Sci.* 922: 352–354

von Hoff, D. D., Layard, M. W., Basa, P. (1979) Risk factors for doxorubicin-induced congestive heart failure. *Ann. Intern. Med.* 91: 710–717

Webb, M. S., Harasym, T. O., Masin, D., Bally, M. B., Mayer, L. D. (1995) Sphingomyelin-cholesterol liposomes significantly enhance the pharmacokinetic and therapeutic properties of vincristine in murine and human tumour models. *Br. J. Cancer* 72: 896–904

Williams, S. S., Alosco, T. R., Mayhew, E., Lasic, D. D., Martin, F. J., Bankert, R. B. (1993) Arrest of human lung tumour xenograft growth in severe combined immunodeficient mice using doxorubicin encapsulated in sterically stabilised liposomes. *Cancer Res.* 53: 3964–3967

Winer, E., Batist, G., Belt, R., Gutheil J., Park, Y., Wells, L. (2000) Reduced cardiotoxicity of liposome-encapsulated doxorubicin (TLC D-99) compared to free doxorubicin in first-line therapy of metastatic breast cancer in patients at increased risk for anthracycline-induced cardiac toxicity. *Proc. Am. Soc. Clin. Oncol.* 19: 64a (abstr. 323)

Wollina, U., Graefe, T., Karte, K. (2000) Treatment of relapsing or recalcitrant cutaneous T-cell lymphoma with pegylated liposomal doxorubicin. *J. Am. Acad. Dermatol.* **42**: 40–46

Wollina, U., Graefe, T., Kaatz, M. (2001) Pegylated doxorubicin for primary cutaneous T-cell lymphoma: a report on ten patients with follow-up. *J. Cancer Res. Clin. Oncol.* **127**: 128–134

Yeo, W., Chan, K. K., Mukwaya, G., Ross, M., Leung, W. T., Ho, S., Chan, A. T., Johnson, P. J. (1999) Phase II studies with Dauno-Xome in patients with non-resectable hepatocellular carcinoma: clinical and pharmacokinetic outcomes. *Cancer Chemother. Pharmacol.* **44**: 124–130

Yip, D., Halford, S., Karapetis, C., Steger, A., Khawaja, H., Harper, P. (1999) A phase II trial of Caelyx™ (liposomal doxorubicin, Doxil™) in the treatment of advanced pancreatic cancer. *Proc. Am. Soc. Clin. Oncol.* **18**: 306a (abstr. 1177)

Zalipsky, S., Qazen, M., Walker, J. A., Mullah, N., Quinn, Y. P., Huang, S. K. (1999) New detachable poly(ethylene glycol) conjugates: cysteine-cleavable lipopolymers regenerating natural phospholipid, diacyl phosphatidylethanolamine. *Bioconjug. Chem.* **10**: 703–707

Zucchetti, M., Boiardi, A., Silvani, A., Parisi, I., Piccolrovazzi, S., D'Incalci, M. (1999) Distribution of daunorubicin and daunorubicinol in human glioma tumors after administration of liposomal daunorubicin. *Cancer Chemother. Pharmacol.* **44**: 173–176